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(54) Title: ANTI-EGFR SINGLE-CHAIN FVS AND ANTI-EGFR ANTIBODIES

(57) Abstract

This invention relates to new anti-EGFR antibodies and single-chain Fvs (scFvs) thereof which can be obtained from phage-antibody libraries constructed from cells of an immunized mammalian, preferably a mouse. Two of the single-chain Fvs isolated from the phage-antibody libraries were engineered to create partially humanized whole antibody molecules. These chimeric anti-EGFR antibodies contain constant regions of human immunoglobulins, and can be used as well as the single-chain Fvs as agents for the diagnosis and therapy of human tumors.

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Anti-EGFR Single-Chain Fvs and Anti-EGFR Antibodies

TECHNICAL FIELD OF THE INVENTION

This invention relates to new anti-EGFR antibodies and antibody fragments, preferrably single-chain Fvs (scFvs) which can be obtained from phage-antibody libraries constructed from cells of an immunized mammalian, preferably a mouse. The antibody fragments isolated from the phage-antibody libraries can be engineered to create partially humanized whole antibody molecules. These chimeric anti-EGFR antibodies contain constant regions of human immunoglobulins, and can be used as well as their fragments as agents for the diagnosis and therapy of human tumors.

Furthermore, the invention demonstrates that phage-antibody libraries are an alternative, and more versatile, method for isolating antibodies from immunized mammalians in comparison with the standard hybridoma technology.

The invention relates, moreover, to pharmaceutical compositions comprising said antibodies or fragments for the purposes of treating tumors like melanoma, glioma or carcinoma. The said antibodies or fragments can be used also for diagnostic applications regarding locating and assessing the said tumors in vitro or in vivo.

The specification relates to several technical terms which are herewith defined as follows:

"FRs" (framework regions) mean the four subregions of the light or heavy chain variable regions that support the three CDRs.

"CDRs" (complementarity determining regions) mean the three subregions of the light or heavy chain variable regions which have hypervariable sequences and form loop structures that are primarily responsible for making direct contact with antigen.

"Chimeric" or partially humanized antibodies mean antibodies comprising constant regions deriving from human sources and variable regions (CDRs included) deriving from non-human sources, e.g. from the mouse.

"Humanized" or fully humanized antibodies mean antibodies comprising constant regions and FRs deriving from human sources whereas the CDRs derive from non-human sources.

"EGF" and "EGFR" mean the epidermal growth factor ant its receptor.

"PCR" means the polymerase chain reaction.

"scFv" means single-chain Fv which is an antibody fragment.

"V_L" means light chain variable region.

"V_k" means kappa light chain variable region.

"V_H" means heavy chain variable region.

PBS means phosphate buffered saline

FCS means fetal calf serum

HBSS means Hanks balanced salt solution

FITC means fluoresceineisothiocyanate

MTC means mixed cell culture

BACKROUND OF THE INVENTION

Epidermal growth factor (EGF) is a polypeptide hormone which is mitogenic for epidermal and epithelial cells. When EGF interacts with sensitive cells, it binds to membrane receptors (EGFR). The EGFR is a transmembrane glycoprotein of about 170 kD and is a gene product of the c-erb-B proto-oncogene.

MAb 425 is a murine monoclonal antibody raised against the well known human A431 carcinoma cell line (ATCC CRL 1555), binds to a polypeptide epitope of the external domain of the human EGFR, and inhibits the binding of EGF. MAb 425 (ATCC HB 9629) was found to mediate tumor cytotoxicity in vitro and to suppress tumor cell growth of epidermoid and colorectal carcinoma-derived cell lines in vitro (Rodeck et al., *Cancer Res.* 1987. *47*: 3692). Humanized and chimeric versions of MAb 425 have been disclosed in WO 92/15683.

Over the last few years, methods have been described (Skerra and Plückthun, *Science* 1988. *240*: 1038; Better et al., *Science* 1988. *240*: 1041) with which functional antibody fragments can be produced in eukaryotic host cells, such as E. coli. These include the Fv fragment and the Fab fragment, whereby the Fv fragment is of special interest. Single-chain Fvs (wherein the V_L and the $V_H$chain are linked together) have been also described (Bird et al., *Science* 1988. *242*: 423; Huston et al., *Proc. Natl. Acad. Sci. USA* 1988. *85*: 5879).

Phage-antibody libraries offer an alternative technology to hybridoma technology in the isolation of antibodies from immunized animals. Hybridoma technology works by immortalizing the cells that produce

the antibodies. Phage-antibody technology works by immortalizing th genes that code for the antibodies (Winter, G. and Milstein, C., *Nature* 1991. *349:* 293). In phage-antibody technology, the antibody heavy chain variable region (V_H) and light chain variable region (V_L) genes are PCR-amplified, the variable regions are randomly combined and expressed as antibody fragments on the surface of phage particles, and libraries of phage antibodies are screened for antibodies that bind to antigens of interest.

Hybridoma technology has been very successful at isolating mouse monoclonal antibodies when it has been possible to generate a strong immune response in the spleens of the animals. For example, mouse MAbs against human epidermal growth factor receptor (EGFR) have been isolated from the spleens of mice immunized intraperitoneally with human A431 tumor cells (Murthy et al., *Arch. Biochem. Biophys.* 1987. *252:* 549). The potential advantage of phage-antibody technology over hybridoma technology is that virtually any source of antibody-expressing cells can be used as starting material and that large numbers of different antibodies can be rapidly screened. Another advantage of the phage-antibody technology is that the genes coding for the variable regions of the antibodies of interest have already been cloned and are immediately available for further genetic engineering.

In one report, an anti-tetanus toxoid Fab fragment isolated from a phage-antibody library was converted into a whole antibody molecule (Bender et al., *Hum. Antibod. Hybridomas* 1993. *4:* 74).

During the last ten years, *in vitro* immunization has been used as an alternative technique to active immunization to generate monoclonal antibodies (mAbs) against a wide variety of antigens from both human

and murine systems (e.g. Vaux, D.J.T.; Helenius, A. and Mellman, I.; Nature, 1988. 336: 36; Gathuru, J.K et al.; J. Immunol. Methods, 1991. 137: 95; Borrebaeck, C.A.K.; Immunol. Today, 1988.9:355). Advantages of this approach are that only small amounts of antigen are required and that the method is applicable for generating human hybridomas. However, the generation of poor affinity IgM antibodies and the difficulty of immortalizing human lymphocytes after *in vitro* immunization have become persistent problems associated with this technology.

A new way of obtaining antibodies is by PCR amplification of repertoires of heavy (V_H) and light (V_L) chain variable region genes which are then randomly recombined and expressed as phage display libraries (7-9). Antibody variable-region genes were cloned and fused to the minor coat protein (gene 3) as a single chain Fv fragment (scFv) (10). The phage particle displays on its surface the antibody fragment and can be selected by panning using the antibody's binding properties. This technology has the advantage that random recombination of V genes may produce novel pairings with new specificities and affinities which could not be selected by natural processes. Moreover, such an approach makes possible the use of naive or *in vitro* immunized lymphocytes from murine or human sources.

Previous attempts to obtain mAbs against EGFR by murine B cells *in vitro* immunization and hybridoma technology rendered low-affinity, cross-reacting antibodies. In order to overcome such handicaps, the combination of *in vitro* immunization followed by PCR cloning technology was carried out.

Therefore, it is an object of the invention to develop antibodies and antibody fragments which have a high affinity to the EGF-receptor and

which can be obtained by the advantageous procedure described above and below.

SUMMARY OF THE INVENTION

This invention compares mouse anti-EGFR antibodies isolated from three different phage-antibody libraries with a mouse MAb (425) isolated by standard hybridoma technology (Murthy et al., *Arch. Biochem. Biophys.* 1987. *252:* 549; Kettleborough et al., *Protein Eng.* 1991. *4:* 773). Libraries were prepared, not only from the spleen of an immunized mouse, but also from the draining lymph node of an immunized mouse and from in vitro immunized mouse cells. Two of the single-chain Fvs (scFvs) that were isolated from the libraries were engineered to create chimeric whole antibody molecules with the mouse variable regions joined to human constant regions.

In detail, the present invention relates to an anti-EGFR single-chain Fv obtainable from phage-antibody libraries constructed from cells, preferrably of the spleen or the draining lymph node of an immunized mammalian, preferably a mouse, or from in vitro immunized cells. In principal, the invention is not restricted to scFvs but extends also to other anti-EGFR antibody fragments such as Fab or $F(ab')_2$.

Some of the scFvs according to the invention have well-defined DNA and amino acid sequences. Therefore, it is another object of the invention to provide a single-chain Fv fragment, wherein the variable

regions of the heavy and light chain comprise a DNA and/or a amino acid sequence selected from one of the heavy and light chain sequences given in Sequence Id. Nos. 1 - 32, preferably in Fig. 5 - 8.

Because in many cases only completely functioning, whole antibodies can be used for diagnostic and therapeutic purposes it is in the interest of the invention to link the variable regions from single-chain Fvs with the constant regions of human immunoglobulins forming whole, partially or humanized anti-EGFR antibodies.

Therefore, it is an object of this invention to provide a whole anti-EGFR antibody constructed from DNA sequences deriving from antibody fragments as defined above, below or as defined in the claims, and from DNA sequences deriving from constant regions of human immunogobulins, wherein, as a preferred embodiment, the heavy chain comprises the amino acid sequence of a gamma-1 chain, and the light chain comprises the amino acid sequence of a kappa chain.

According to the present invention the anti-EGFR scFvs, are isolated by using the phage-antibody library technology. Therefore the invention relates to a process for the preparation of an anti-EGFR single-chain Fv comprising the following steps:

(i) isolating RNA from immunized mammalian cells, preferably mouse cells,

(ii) synthesizing first-strand cDNA,

(iii) amplifying the V_H and V_k genes in cDNAs from the immunized cells,

(iv) cloning said genes together with suitable restriction sites into a phagemid vector,

(v) transforming prokaryotic cells with the ligation mixtures,

(vi) screening the phage libraries for phage antibodies directed to EGFR using purified EGFR, and

(vii) producing the desired single-chain Fv in prokaryotic host cells, preferably E. coli.

Additionally, it is an object of the invention to disclose a process for the preparation of a whole anti-EGFR antibody by cloning the DNA coding for the variable regions of anti-EGFR antibody fragments produced as indicated above or as defined in the claims into at least one eukaryotic expression vector containing genomic DNA which codes for the constant regions of human immunoglobulins, transforming eukaryotic cells with said vector(s) and expressing and isolating the antibody.

The anti-EGFR scFvs, and above all, the whole anti-EGFR antibodies can be used in diagnosis and therapy of human tumors. Thus, the invention relates to a pharmaceutical composition comprising an anti-EGFR single chain Fv or a whole anti-EGFR antibody as defined above or as defined in the claims.

The results and advantages of the present invention can be summarized as follows:

Novel mouse anti-EGFR antibodies were isolated from phage-antibody libraries. The new antibodies represented at least four different $_{VH}$ subgroups and four different $V_K$subgroups (Kabat et al., *Sequences of proteins of immunological interest*. 5th Eds., U.S. Dept. of Health and Human Services, Bethesda 1991). They showed different pairings and sequences from those used by a mouse MAb isolated using hybridoma technology. Mouse 425 MAb has a $_{VH}$2b and V_K4 pairing that was not

observed in the phage antibodies. The $_{VH}$ of scFv L3 11D had the highest percent identity to 425 $_{VH}$ (84.9%). The majority of the differences were in the CDRs. The V_k of scFv S4 2D had the highest percent identity to 425 V_k (83.2%). Again the majority of differences were in the CDRs, particularly CDR3. In this invention, a variety of new anti-EGFR antibodies were isolated from the phage-antibody libraries and these antibodies all differ from 425 MAb with at least two of the scFvs recognizing a different epitope on EGFR from that recognized by 425 MAb. This is in contrast to a previous report where the antibodies isolated from combinatorial libraries were reported to be very similar to those isolated by hybridoma technology (Caton and Koprowski, *Proc. Natl. Acad. Sci. USA* 1990. *87:* 6450).

Of the three phage-antibody libraries, the best library in terms of the number of selection steps required to get high affinity antibodies and in terms of the diversity of high affinity antibodies isolated was the library generated from the draining lymph node. Lymph nodes were selected as a source of RNA for the construction of phage-antibody libraries for two reasons. First, previous work had demonstrated that a higher proportion of B cells producing high affinity IgG antibodies was obtained from the popliteal lymph nodes following immunization via the footpad than from spleens following immunization via the peritoneum (Venn and Dresser, *J. Immunol. Methods* 1987. *102:* 95). Second, draining lymph nodes are considered to be a good source for the isolation of human anti-tumor antibodies. Thus, the isolation of mouse anti-EGFR antibodies from the popliteal lymph node of a footpad-immunized mouse was a model for the isolation of human anti-EGFR antibodies from the axillary lymph nodes of a breast cancer patient. The feasibility of preparing good size libraries from small amounts of

lymph node material and of then isolating high affinity antibodies from the libraries was demonstrated.

Although mouse anti-EGFR antibodies were isolated from all three phage-antibody libraries, it is not clear that any of the newly isolated antibodies have higher affinities than the mouse 425 MAb isolated using hybridoma technology. In the first analyses, the phage-antibody derived scFvs appeared to bind to EGFR better than the scFv constructed from 425 MAb (Fig. 2). In other experiments with chimeric whole antibody molecules, one of the chimeric antibodies (S4 2D) showed an affinity for EGFR that equalled that of the chimeric 425 antibody. The second chimeric antibody (L3 11D) had an affinity that was four-fold lower than that of chimeric 425 antibody (Fig. 4). Binding data obtained using scFvs was misleading probably because preparations of scFvs can contain mixtures of monomers and dimers (Griffiths et al., *EMBO J.* 1993. *12:* 725). In contrast, chimeric IgG antibodies are not expected to form dimers and the chimeric L3 11D and S4 2D antibodies were demonstrated to be the size expected for bivalent, monomeric chimeric IgG antibodies. Analysis of affinity-purified preparations of 425, L3 11D, and S4 2D scFvs, however, showed that these preparations of scFvs did contain monomeric, dimeric, and other multimeric forms. In addition, the relative proportions of monomeric and multimeric forms varied for each scFv. The 425 scFv had the lowest percentage of dimeric forms. As predicted, the dimeric and particularly the larger multimeric forms showed stronger binding to purified EGFR than the monomeric form. It appears that 425 scFv has a weaker tendency to dimerize than some of the newly isolated scFvs.

Although the expression of antibody fragments on the surface of phage particles forms the basis of a powerful method for rapidly selecting for

antibodies with the desired specificities, neither phage antibodies nor the antibody fragments themselves (scFvs or Fabs) are likely to be the desired end product. Further it is demonstrated how the mouse scFvs isolated from phage libraries can be readily converted into whole antibody molecules. In this case, the mouse variable regions were joined to human constant regions to create partially humanized chimeric antibodies.

These results show that it is possible to use phage-antibody technology to isolate a variety of anti-EGFR antibody fragments from immunized mice. Whole antibody molecules with the desired constant regions can then be constructed from the antibody fragments. In some cases, hybridoma technology may still be the method of choice for isolating monoclonal antibodies from mice. If a highly immunogenic antigen is available and if a few hybridoma cell lines producing one or a few different anti-antigen antibodies are adequate, then there is probably little reason to consider phage-antibody technology. If, however, special immunization protocols such as footpad injections would be advantageous in generating high affinity antibodies, or if a large number of antibodies against a variety of epitopes on the antigen are required, or if antibodies directed against a very discreet, and possibly less immunogenic, epitope are required, then phage-antibody technology may be the method of choice. Also, if further genetic engineering of the antibodies is anticipated, then the phage-antibody technology is advantageous in that the antibody genes have already been cloned.

The present approach of combining *in vitro* immunization with a particulate antigen and PCR-cloning technology has generated scFv fragments which reacted with EGFR and did not cross-react with other antigens. The immunization protocol reported here depends on the

antigen presentation, which is not soluble but is a membrane vesicle preparation, and on the culture medium itself, which is devoid of FCS. Both methodologies have been reported as a means of increasing the efficiency of *in vitro* immunization by making the antigen available to the antigen-presenting cells (e.g. Brams, P. et al.; J. Immunol. Methods, 1987. 98: 11).

The results obtained with MTC are in agree with previous papers (e. g. Borrebaeck, C.A.K. and Möller, S.A.; J. Immunol., 1986. 136: 3710; .Möller, S.A. and Borrebaeck, C.A.K., in Borrebaeck, C.A.K. (Eds.), In Vitro Immunization in Hybridoma Technology, Elsevier Science Publishers B.V., Amsterdam 1988, p. 3.) which propose the use of MTC supernatants as a source of lymphokines for improving the *in vitro* immunization process. The membrane vesicle preparation should be envisaged as a poly-antigen since many different antigenic determinants are present in such vesicles. For this reason, it would appear that they induce a certain level of polyclonal activation. We have ruled this out because the anti-EGFR specific response was clearly different from the response obtained after a standard polyclonal activator.

Instead of immortalizing the B-cells after *in vitro* immunizations, we have used the molecular strategy of immortalizing the antibody V_H and V_L genes. These monoclonal antibody fragments were expressed and produced in bacteria. The phage display system is a powerful method to isolate antibody fragments against specific antigens. The presence of a stop codon between the antibody fragment and the g3p coat protein permits the switch between surface display and secretion as a soluble scFv fragment using suppressor or non-suppresor strains (Hoogenboom et al., Nucl. Acids Res. 1991. 19: 4133).

Due to the increase of specific response and mRNA levels in *in vitro* antigen stimulated B-cells, *in vitro* immunization contributes to the

isolation of antibody fragments with high specificities to the antigen. After two rounds of selection, 100% of the clones were positive for binding EGFR. In contrast, clones derived from *in vivo* immunization processes were 100 % positive only after four rounds of selection (Kettleborough, et al., EP 94104160 and Eur. J. Immunol. 1994. 24: 952).

The use of phage display libraries from naive antibody genes might allow specific human antibody fragments to be made without immunization or after *in vitro* immunization. Antibody fragments can be directly produced in bacteria, thus in a simple, fast and economic way.

BIOLOGICAL MATERIALS AND GENERAL METHODS

Microorganisms, cell lines, plasmids, phagemids, promoters, resistance markers, replication origins or other fragments of vectors which are mentioned in this application are commercially or otherwise generally available. Provided that no other information in the application is given, they are used only as examples and are not essential according to the invention and can be replaced by other suitable tools and biological materials, respectively.

Bacterial hosts are preferably used for cloning the scFvs and for producing the scFv proteins. Examples for these hosts are: E. coli or Bacillus.

Eukaryotic hosts like COS, CHO or yeasts, for example, are preferred in order to produce the whole anti-EGFR-antibodies according to the invention.

The techniques which are essential according to the invention are described in detail in the specification. Other techniques which are not described in detail correspond to known standard methods which are well known to a person skilled in the art, or are described more in detail in the cited references and patent applications and in the standard literature.

Brief Description of the Figures:

Figure 1. Amino acid sequences of scFvs isolated from phage-antibody libraries. (A) scFvs from the lymph node library. (B) scFvs from the spleen library. Complementarity determining regions (CDRs) and framework regions (FRs) are indicated.

Figure 2. Binding of scFvs to EGFR. The concentrations of scFvs in bacterial supernatants were estimated and the scFvs tested by ELISA for binding to purified EGFR. (A) scFvs from the lymph node library. (B) scFvs from the spleen library. P1 (positive control) is the scFv derived from MAb 425. L1 and S1 (negative controls) are non-binding scFvs from the pre-selected lymph node and spleen libraries.

Figure 3. Intermediate vectors used to reconstruct the variable regions for expression in mammalian cells. (A) V_H vector. (B) V_K vector.

Figure 4. Binding of chimeric whole antibodies to EGFR. The concentrations of antibodies in COS cell supernatants were determined by ELISA and the antibodies tested by ELISA for binding to purified EGFR.

Figure 5. DNA and amino acid sequence of scFv No. L2 11C.
(A): Light chain; (B): Heavy chain.
Amino acid positions:

(A) FR-1: 1 - 23, CDR-1: 24 - 34,
FR-2: 35 - 49, CDR-2: 50 - 56,
FR-3: 57 - 88, CDR-3: 89 - 97,
FR-4: 98 - 109.

(B) FR-1: 1 - 30, CDR-1: 31 - 35,
FR-2: 36 - 49, CDR-2: 50 - 66,
FR-3: 67 - 98, CDR-3: 99 - 108,
FR-4: 109 - 119.

Figure 6. DNA and amino acid sequence of scFv No. L2 12B.
(A): Light chain; (B): Heavy chain.
Amino acid positions:

(A) FR-1: 1 - 23, CDR-1: 24 - 38,
FR-2: 39 - 49, CDR-2: 50 - 56,
FR-3: 57 - 88, CDR-3: 89 - 97,
FR-4: 98 - 109.

(B) FR-1: 1 - 30, CDR-1: 31 - 35,
FR-2: 36 - 49, CDR-2: 50 - 66,
FR-3: 67 - 98, CDR-3: 99 - 108,
FR-4: 109 - 119.

Figure 7. DNA and amino acid sequence of scFv No. L3 11D.
(A): Light chain; (B): Heavy chain.
The amino acid posititions of the FRs and CDRs correspond to those given in Fig. 6.

Figure 8. DNA and amino acid sequence of scFv No. S4 2D
(A): Light chain; (B): Heavy chain.
Amino acid positions:

(A) FR-1: 1 - 23, CDR-1: 24 - 35,
FR-2: 36 - 50, CDR-2: 51 -57,
FR-3: 58 - 89, CDR-3: 90 - 98,
FR-4: 99 - 110

(B) FR-1: 1 - 30, CDR-1: 31 - 35,
FR-2: 36 - 49, CDR-2: 50 - 66,
FR-3: 67 - 98, CDR-3: 99 - 107,
FR-4: 108 - 118.

The sequences of Figures 5 - 8 are also given in the attached Sequence Listing which is part of the disclosure of this invention.

DETAILED DESCRIPTION OF THE INVENTION

(1) Construction and screening of phage-antibody libraries

Three phage-antibody libraries were constructed, one from the spleen of a mouse immunized with human carcinoma cell line A431 (8.8×10^5 members), one from the popliteal lymph node of a mouse immunized in the footpad with purified EGFR (6.5×10^6 members), and one from mouse lymphocytes immunized *in vitro* with A431 vesicles (1.1×10^5 members), (details of construction of A431 vesicles and *in vitro*-immunization are given in Examples 1, 2). Prior to selection, at least 46 clones from each library were analyzed by BstNI fingerprinting

(Clackson t al, *Nature* 1991. *352:* 624) to determine the diversity of the repertoires. A wide range of digestion patterns was observed. Also prior to selection, scFvs from 96 clones from each library were tested by ELISA for binding to EGFR. None of the scFvs from the spleen and lymph node library bound to EGFR. One of the scFvs from the *in vitro* immunized library bound to EGFR. After one round of selection using EGFR-coated immunotubes, a clear enrichment for EGFR-binding scFvs was observed with the lymph node library and with the *in vitro* immunized library. A second round of selection was needed before any EGFR-binding scFvs were detected from the spleen library. By the third round of selection, the majority of the scFvs from the lymph node and *in vitro* immunized libraries were positive for binding to EGFR. After a fourth round of selection with the spleen library, the majority of the scFvs were positive for binding to EGFR (Table 1).

Table 1. Percent of EGFR-binding clones after each round of selection.

	Lymph Node Library	Spleen Library	*In vitro* Immunized Cells Library
Pre-selection	0	0	1
First round	77	0	84
Second round	86	26	100
Third round	90	77	100
Fourth round	not tested	97	not tested

(2) Sequence analysis of EGFR-binding clones

After each round of selection, scFv inserts from EGFR-binding clones were analyzed by BstNI fingerprinting (Clackson et al, *Nature* 1991. *352:* 624). It became clear that there was an enrichment for certain digestion patterns. Clones with different BstNI fingerprints were chosen from the second and third rounds of selection of the lymph node library and from the third and fourth rounds of the spleen library for DNA sequencing of the V_Hs and V_Ks. Clones from later rounds of selection

were analyzed because higher affinity antibodies were expected to be in the later rounds (Clackson et al, *Nature* 1991. *352:* 624).

Sixteen clones from the lymph node library were sequenced and six different scFvs were obtained (Figure 1). Five of these were pairings of unique V_Hs and V_κs. The sixth was a variation of a previously occurring V_H with six amino acid changes, five of which were in framework region (FR) 1. Two of these changes can be attributed to the use of the degenerate VH1BACKSFI primer (Hoogenboom et al., *Nucl. Acids Res.* 1991. *19:* 4133). The others may be a result of PCR errors. The V_Hs were classified into two subgroups, V_H2b and V_H3d, while the V_κs fell into four subgroups, $V_\kappa3$, $V_\kappa4$, $V_\kappa5$, and $V_\kappa6$ (Kabat et al., *Sequences of proteins of immunological interest.* 5th Eds., U.S. Dept. of Health and Human Services, Bethesda 1991). Ten individual clones from the spleen library were sequenced and four different scFvs were found. Three of these were pairings of unique V_Hs and V_κs while the fourth was similar to one of the previous pairings with only two amino acid differences in V_H, one of which occurred in complementarity determining region (CDR) 2, and two amino acid differences in V_κ. Classification into subgroups revealed V_Hs from subgroups V_H2a, V_H2c, and V_H3d and V_κs from subgroups $V_\kappa3$ and $V_\kappa4$. Comparison of the scFvs obtained from the lymph node and spleen libraries revealed only one scFv that was common to both libraries, scFv L3 10A/scFv S4 10H (Figure 1). This clone appeared to bind strongly to EGFR when tested by ELISA. While much care was taken to eliminate any cross-contamination between libraries, it is difficult to rule out minor contamination with a strongly-binding EGFR clone. However, considering the inbred nature of Balb/c mice, it is possible that the same scFv arose independently from two different libraries.

(3) Analysis of the affinity and specificity f binding to EGFR

Based on good binding to antigen and diversity in DNA sequences, several scFvs derived from the lymph node and spleen libraries were chosen for further analysis. These scFvs were analyzed by ELISA for binding to purified EGFR, binding to irrelevant antigens, and binding to tumor cell lines that did or did not express EGFR. As a positive control, scFvs were prepared from mouse 425 MAb (P1). As negative controls, scFvs were prepared from phage antibodies isolated from the lymph node and spleen libraries prior to selection (L1 and S1, respectively). The concentration of scFvs was determined by comparing dilutions of the scFvs to be tested with dilutions of a purified scFv of known concentration in a Western blot.

The scFvs were tested by ELISA for binding to purified EGFR and the results plotted (Figure 2). It was possible to rank the scFvs with respect to their binding to EGFR. These rankings were reproducible between experiments. The scFvs that bound most strongly to EGFR were L2 1C and L3 10A from the lymph node library and S4 10H from the spleen library. As described previously, scFvs L3 10A and S4 10H have the same DNA sequences. A scFv (S4 5A) that was very similar to scFv S4 10H, with two amino acid changes in V_H and two in V_k, consistently gave a lower ranking than S4 10H. In contrast, the differences in sequence observed between L2 12B and L3 11D did not appear to have a pronounced effect on the binding. Of the scFvs isolated only two, L2 8C and L2 11C, appeared to bind less well than scFv 425.

The scFvs were tested by ELISA for binding to plastic and to a panel of unrelated proteins (ovalbumin, hen egg lysozyme, cytochrome c, glyceraldehyde 3-phosphate dehydrogenase, CBA albumin, and BSA). None of the scFvs gave a signal above background.

The scFvs were tested by ELISA for binding to three tumor cell lines. Cell lines A431 and MDA MB 468 are EGFR-bearing tumor cells isolated from the vulva and breast, respectively. Cell line SK-MEL-23 is a ganglioside-bearing melanoma cell line and was included as a negative control. Of the ten scFvs tested, only four bound to both purified EGFR and EGFR-bearing tumor cells (L2 12B, L3 11D, L2 11C, and S4 2D, Figures 5 - 8). No binding to SK-MEL-23 cells was detected. There are several possible explanations for this surprising result. One may be that the EGFR that was used for immunization, selection, and ELISA was secreted EGFR-related protein (Weber et al., *Science* 1984. *224:* 294). This protein has an additional 17 amino acids at the C-terminus (Günther et al., *J. Biol. Chem.* 1990. *265:* 22082). The scFvs were tested by ELISA for binding to this 17 amino acid peptide and no binding was observed. It is possible that the secreted EGFR-related protein and the EGFR on the tumor cell surface have differences in conformation or glycosylation.

To further investigate binding to tumor cells, three scFvs (L2 11A, L3 11D and S4 2D) were purified and analyzed for binding to A431 tumor cells by flow cytometry. The 425 scFv was used as a positive control. Of the three scFvs tested, only L3 11D and S4 2D bound to A431 cells. These two scFvs had similar binding profiles to scFv 425.

Purified scFvs prepared from two of the isolates that bound to both EGFR and EGFR-bearing tumor cells (L3 11D and S4 2D) were tested in competition binding assays with mouse 425 MAb. While purified scFv 425 was able to inhibit mouse 425 MAb from binding to EGFR over a given concentration range, scFvs L3 11D and S4 2D did not inhibit mouse 425 MAb from binding to EGFR at these concentrations. These two scFvs appear to recognize an epitope on EGFR that is different from that recognized by mouse 425 MAb.

(4) Chimeric whole antibodies derived from scFvs.

Two scFvs (L3 11D and S4 2D) were selected for conversion into whole antibody molecules. DNAs coding for the mouse V_Hs and V_Ks were cloned into intermediate vectors containing DNA sequences coding for immunoglobulin leader sequences and splice donor signals (Fig. 3). The positioning of the cloning sites in the V_H intermediate vector meant that the first residue of the V_H was changed from aspartic acid to glutamic acid. From the intermediate vectors, DNA fragments containing the V_Hs and V_Ks, now joined to leader and splice donor sequences, were cloned into mammalian cell expression vectors containing DNAs coding for either human gamma-1 constant region or human kappa constant region (Maeda et al., *Hum. Antibod. Hybridomas* 1991. *2:* 124). For each chimeric antibody, the heavy chain and light chain expression vectors were co-transfected into COS cells. As a positive control, cells were also co-transfected with heavy and light chain expression vectors coding for chimeric 425 antibody (Kettleborough et al., *Protein Eng.* 1991. *4:* 773). Medium was collected from the cells and analyzed by ELISA to determine the concentration of antibody present and the ability of the antibody to bind to EGFR (Fig. 4). When the antibody concentration required to achieve half-maximum binding to antigen were compared, chimeric S4 2D antibody bound to EGFR equally as well as chimeric 425 antibody. Chimeric L3 11D antibody, however, bound to EGFR approximately four-fold less well than chimeric 425 antibody. The affinity of chimeric 425 antibody (Kettleborough et al, *Protein Eng.* 1991. *4:* 773) has been determined by competition binding analysis to be 1.9×10^8 M^{-1}. These results were surprising because previous data analyzing the scFvs had indicated that scFvs S4 2D and L3 11D both bound to EGFR better

than scFv 425 (Fig. 2). Protein A-purified samples of chimeric L3 11D and S4 2D antibodies were analyzed by SDS-PAGE under reducing and non-reducing conditions. Chimeric L3 11D and S4 2D antibodies were also tested by flow cytometry for binding to A431 and SK-MEL-23 cells. Both chimeric antibodies bound well to the EGFR-expressing A431 cells and did not bind to the EGFR-negative SK-MEL-23 cells.

(5) Therapeutic and diagnostic use

The antibody fragments and whole antibodies according to the invention can be administered to human patients for therapy. Therefore, it is an object of the invention to provide a pharmaceutical formulation comprising as active ingredient at least one antibody or antibody fragment as defined above and in the claims, associated with one or more pharmaceutically acceptable carrier, excipient or diluent therefore.

Typically the antibody of this invention will be injected intravenously or parenterally. Generally, the dosage ranges for the administration of the antibodies fragments are large enough to produce the desired tumor suppressing and tumor lysing effect. The dosage will depend on age, condition, sex and extent of the disease in the patient and can vary from 0.1 mg/kg to 200 mg/kg, preferably from 0.1 mg/kg to 100 mg/kg/dose in one or more doses administered daily, for one or several days.

Preparations for parenteral administration includes sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oils, and injectable organic esters such as ethyl oleate and other solvents known in the art which are suitable for these

purposes. Th antibodies of this invention can be used in a composition comprising a physiologically acceptable carrier. Examples of such suitable carriers are saline, PBS, Ringer's solution, or lactated Ringer's solution. Preservatives and other additives such as antibiotics, antioxidants, and chelating agents may also be present in the pharmaceutical formulations.

The antibody (fragment) can also be conjugated according to known methods to cytokines such as IL-2 in order to support their cytotoxicity.

The pharmaceutical formulations of the present invention are suitable for the treatment of all kinds of tumors, including melanomas, gliomas and carcinomas, as well as tumors of the circulating system and solid tumors.

For diagnostic purposes the antibody can be conjugated, for example, to a radio-opaque dye or can be radiolabelled. A preferred labelling method is the Iodogen method. Preferably the antibody will be administered as $F(ab')_2$ or scFv fragments for diagnostic purposes. This provides superior results so that backround substraction is unnecessary.

EXAMPLE 1: A431 vesicles

Shed membrane vesicle preparations were obtained as previously described by (Cohen et al., *J. Biol. Chem.* 1982. *257*: 1523; Yeaton et al., *J. Biol. Chem.* 1983. *258*: 9254) with some modifications. Confluent flasks containing A431 cells were washed with PBS containing calcium

and magnesium. Hypotonic PBS was added and flasks were shaken for 15 minutes. Cells were then washed with vesiculation buffer (100 mM NaCl, 50 mM Na_2HPO_4, 5 mM KCl, 0.5 mM $MgSO_2$, pH 8.5). Vesiculation buffer was added and flasks were kept in agitation at room temperature and at 37°C. Then, buffer was decanted through metallic screen into 50 ml tubes in ice and centrifuged for 5 minutes at 150 x g at 4°C. The pellet was discarded and the supernatant was ultracentrifuged at 39,000 rpm for 90 minutes. The final pellets were resuspended in 10 mM Hepes buffer (pH 7.4). To analyze EGFR from vesicles, samples were precipitated with 9 volumes of ethanol resuspended with 0.08 M Tris, pH 6.8, and then SDS-PAGE was carried out with MAb 425 as standard.

The protein content of the preparations was quantitated by a modified Coomassie Plus method using BSA as a standard and read at 595 nm. To analyze EGFR from vesicles, samples were precipitated with 9 volumes of ethanol (overnight at 4°C). The pellet was resuspended with Tris (0.08 M, pH 6.8) and then a SDS-PAGE was run (5% stacking gel; 1h, 35 mA; 10% running gel; 2.5 h; 40 mA). Samples and standard were in duplicate. One of them was stained with Coomassie Blue and the other was blotted onto nitrocellulose sheets (12 V; 16 h at 4°C) and treated with mouse mAb 425 (anti-EGFR) and anti-mouse IgG antibody conjugated to alkaline phosphatase.

Three media were used in the *in vitro* immunizations. Medium-1 (M1), Medium-2 (M2) and Mixed Thymocyte Culture medium (MTC). M1 consisted of HL1 (Ventrex Laboratories, USA) supplemented with 50 mM. 2-mercaptoethanol and 2 mM L-Glutamine (Gibco). M2 consisted of HL1 supplemented with 50 mM 2-mercaptoethanol; 40 U/ml IL-2 (Genzyme); 20 mg/ml Adjuvant Peptide (Sigma); 2 mM L-glutamine; 100 U/ml penicillin (Gibco); 100 mg/ml streptomycin (Gibco). 4% or 20% of FCS

(Biological Industries) was added to M2. MTC was prepared as described by Vaux (1). Briefly single cell suspensions of thymuses of three-week-old Balb/c and C57/BL-1 mice were prepared by pressing the thymus glands through a sterile 50-mesh screen. The cell sus-pension was collected, washed twice in HBSS and the number of viable cells was determined by trypan blue exclusion. Thymocytes were then cultured at a density of $2.5x10^6$ thymocytes of each strain per ml in HL1 medium containing 4% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. After 48 hours, the supernatant was recovered, filtered through a 0.22 mm filter, and stored at -70°C.

A suspension of splenocytes from non-immunized eight-week old BALB/c mice was obtained as described for thymocytes. Viability was determined by trypan blue exclusion.

EXAMPLE 2: In vitro immunization and screening

Three media were used in the *in vitro* immunizations. Medium-1 (M1), Medium-2 (M2) and Mixed Thymocyte Culture medium (MTC). M1 consisted of HL1 (Ventrex Laboratories, USA) supplemented with 50 mM 2-mercaptoethanol and 2 mM L-Glutamine (Gibco). M2 consisted of HL1 supplemented with 50 mM 2-mercaptoethanol; 40 U/ml IL-2 (Genzyme); 20 mg/ml Adjuvant Peptide (Sigma); 2 mM L-glutamine; 100 U/ml penicillin (Gibco); 100 mg/ml streptomycin (Gibco). 4% or 20% of FCS (Biological Industries) was added to M2. MTC was prepared as described by Vaux (1). Briefly single cell suspensions of thymuses of three-week-old Balb/c and C57/BL-1 mice were prepared by pressing the thymus glands through a sterile 50-mesh screen. The cell sus-pension was collected, washed twice in HBSS and the number of viable cells was determined by trypan blue exclusion. Thymocytes were then cultured at a density of $2.5x10^6$ thymocytes of each strain per ml in HL1 medium

containing 4% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. After 48 hours, the supernatant was recovered, filtered through a 0.22 mm filter, and stored at -70°C.

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Single cell suspensions from thymuses of three-week-old Balb/c and C57/BL-1 mice were obtained by pressing the thymus glands through a sterile 50-mesh screen. The cell suspension was collected, washed with HBSS and the number of viable cells was determined by trypan blue exclusion. Thymocytes were then cultured at a density of $2.5x10^6$ thymocytes of each strain per ml in HL1-medium containing 4% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. After 48 hours the supernatant was recovered, filtered and stored. A suspension of splenocytes from non-immunized eight-week-old BALB/c mice was obtained as described for thymocytes. Viability was determined by trypan blue exclusion.

In vitro immunizations were performed in 6-well plates (Costar). Wells containing 10^7 splenocytes in 3.5 ml of M1-medium (consisting of HL1-medium, Ventrex Laboratories, USA, supplemented with 50 µM 2-mercaptoethanol and 2 mM L-glutamine (Gibco)) were incubated (37°C, 5% CO_2) with vesicles bearing EGFR at the desired concentration. Vesicles from cells not expressing EGFR or PBS were added in control wells. After some hours , 3.5ml of M2-medium (consisting of HL1 supplemented with 50 µM 2-mercaptoethanol, 40 U/ml IL-2 (Genzyme), 20 µg/ml adjuvant peptide (SIGMA), 2 mM L-glutamine, 100 U/ml penicillin (Gibco), 100 µg/ml streptomycin (Gibco)) containing 4% or 10% FCS (Biological Industries) was added to each well. In some experiments M2 was replaced by MTC-medium

(mixed thymocyte culture medium (Vaux et al., Nature 1988. 336: 36) supplemented with adjuvant peptide (20µg/ml) and IL-2 (40 U/ml) (Note that the final concentration of FCS, IL2 and adjuvant peptide in culture is 50% reduced). Cells were incubated for 72, 96, 120 or 144 h in the same conditions and, finally, the cells were tested for the presence of specific immunoglobulin or processed for RNA isolation.

Screening was carried out with purified antigens or A431 fixed cells. The procedure was essentially as previously described (Carroll et al., *Hybridoma* 1990. 9: 81) with some modifications. Briefly, sterile 96-well plates (Nunc, Maxisorb) were coated overnight with purified EGFR (2.5 µg/ml), GD_3 ganglioside (2µg/ml), or RNase (10µg/ml) in PBS. When A431 cells were used as antigen, cells were cultured in 96-well plates until confluent and fixed with 0.1 % glutaraldehyde. *In vitro* immunized lymphocytes were washed and resuspended in HL1 medium supplemented with 2 % FCS and 2 mM of L-glutamine at $5x10^5$ cells/ml and $1x10^5$ cells were added to each well and incubated (37°C, 5% CO_2) for 48 h. Sixteen duplicates of each group were done. Lymphocytes were then removed by washing 5 times in PBS containing 0.1 % Tween-20. Specific immunoglobulins were detected using peroxidase labelled rabbit anti-mouse immunoglobulin (Dako) (1 hour, 37°C). 2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulfonicacid)-diammonium salt (ABTS) (Sigma) in citrate-phosphate buffer (0.55 mg/ml) was used as substrate.

EXAMPLE 3: Library construction

Three libraries were constructed from RNA prepared from the spleen of a mouse immunized intraperitoneally with A431 cells (Murthy et al., *Arch. Biochem. Biophys.* 1987. 252: 549) from the popliteal lymph node of a mouse immunized in the footpad with purified EGFR, and from mouse cells immunized *in vitro* with A431 vesicles. First-strand

cDNA was synthesized. The V_H and V_κ genes were PCR-amplifi d and assembled (Clackson et al., *Nature* 1991. *352:* 624). Using PCR, NotI and SfiI restriction sites were appended and the scFvs cloned into the phagemid vector pHEN1 (Hoogenboom et al., *Nucl. Acids Res.* 1991.*19:* 4133). The ligation mixtures were electroporated into *E. coli* cells and the resulting colonies scraped into medium to generate library stocks (Marks et al., *J. Mol. Biol.* 1991. *222:* 581).

EXAMPLE 4: Library screening

Phage antibodies were rescued from the libraries using M13K07 helper phage (Promega, Madison, WI) (Marks et al., *J. Mol. Biol.* 1991. *222:* 581). Immunotubes (Nunc, Life Sciences, Paisley, UK) were coated with 4 ml of 2.5 µg/ml EGFR in PBS overnight. After three washes with PBS, tubes were incubated at 37°C for at least 1 h in PBS containing 2% milk powder (PBSM). The phage (10^{12} to 10^{13}) were resuspended in 4 ml PBSM and incubated in the EGFR-coated tube for 1 h at room temperature. The tube was washed 20 times with PBS, 0.1% Tween and 20 times with PBS. Bound phage were eluted after a 10 min incubation in 1 ml of 0.1 M triethylamine with end-over-end mixing. The eluted phage were neutralized by the addition of 0.5 ml of 1 M Tris-HCl, pH 7.5 and used to infect log-phase *E. coli* TG1 cells. Infected cells were plated and individual colonies picked for small-scale induction of scFvs. The remaining colonies were scraped into medium and an aliquot used to prepare phage for the next round of screening.

EXAMPLE 5: Production and analysis of scFvs

Soluble scFvs were produced in *E. coli* HB2151 as previously described (e.g. Kettleborough et al., l. c.). The scFv concentrations in the bacterial supernatants were estimated using a purified scFv preparation of known concentration as a standard. Supernatants were filtered and sodium azide added to 0.1%. Serial dilutions of the supernatants and of the standard were spotted onto Immobilon-PVDF filters (Millipore, Watford, UK) using a 96-well manifold. The filters were treated as for a Western blot (Towbin et al., *Proc. Natl. Acad. Sci. USA* 1979. *76:* 4350). The scFvs were detected using an antibody (9E10) directed against the C-terminal tag (Munro and Pelham, *Cell* 1986. *46:* 291) followed by a peroxidase-conjugated goat anti-mouse IgG and IgM antibody (Jackson ImmunoResearch Lab Inc., West Grove, PA). The reactions were developed using the ECL system (Amersham, Aylesbury, UK). Pre-flashed autoradiographs were scanned using a densitometer. A standard curve was prepared and used to estimate the scFv concentrations in the supernatants.

Antigen-binding ELISAs were carried out with EGFR-coated plates (2.5 µg/ml). Supernatants containing scFvs were diluted in PBSM and added to the plates. Bound scFvs were detected using 9E10 antibody as described above. Supernatants were also tested for binding to a panel of unrelated proteins and plastic. ELISA plates were coated overnight at 100 µg/ml with ovalbumin, hen egg lysozyme, cytochrome c, glyceraldehyde 3-phosphate dehydrogenase, murine albumin (CBA strain), and BSA. Undiluted supernatants containing 2% milk powder were added in duplicate to the coated plates and bound scFvs detected as described above.

Cell-binding ELISAs were carried out using tumor cell lines, A431 (ATCC CRL 1555), MDA MB 468 (ATCC HTB 132), and SK-MEL-23 (negative control). Cells were grown to confluency in poly-D-lysine-

treated 96 well tissue culture trays (Nunc). C lls were washed with DMEM and blocked at 37°C for 2 h with PBS containing 2.5% BSA. After aspiration, supernatants were added to each well together with an equal volume of 2xYT media containing 4% milk powder and incubated at 4°C for 1 h. Bound scFvs were detected as described above.

A competition-based ELISA was carried out by pre-incubating EGFR-coated ELISA plates with 50 µl of purified scFv (100 µg/ml) for 10 min. Mouse MAb 425 (50 µl) was then added to give concentrations of 3.13 to 200 ng/ml. Following incubation and washing, bound mouse MAb 425 was detected using peroxidase-conjugated goat anti-mouse IgG and IgM antibody.

EXAMPLE 6: DNA analysis

For BstNI fingerprinting, the scFv inserts from individual clones were amplified by PCR and the products digested with BstNI (Clackson et al., *Nature* 1991. *352:* 624). DNA was sequenced using a Sequenase kit (United States Biochemical, Cleveland, OH).

EXAMPLE 7: Purification of scFvs

Bacterial supernatants were clarified by centrifugation and filtration through 0.2µm filters before loading onto a 1 ml column of purified EGFR (5 mg) coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The column was washed with 30 ml of PBS followed by 5 ml 0.2M glycine, pH 5.0. The scFvs were eluted

with 0.2M glycine/HCl, pH 2.8. The eluate was neutralized with 10x PBS. Protein-containing fractions were pooled and the buffer changed by ultrafiltration (Amicon, Stonehouse, UK) to PBS containing 1% BSA and 0.05% sodium azide.

EXAMPLE 8: FACS analysis of purified scFvs

A431 cells were trypsinized and incubated in DMEM containing 10% FCS. Cells were washed twice with cold DMEM and filtered through a 45 µm screen. Cells (10^6) were incubated on ice for 30 min in 50 µl PBS, 1% BSA, with purified scFvs. After two washes with cold PBS, bound scFvs were detected using 50 µl FITC-conjugated 9E10 antibody (100 µg/ml). After 30 min on ice, cells were washed once with PBS, fixed in PBS containing 1% formaldehyde, and analyzed using a FACSCAN (Becton-Dickinson, Cowley, UK).

EXAMPLE 9: Construction, analysis and expression of whole chimeric antibodies

Using PstI and BstEII sites, DNAs coding for the V_Hs of the selected scFvs were subcloned into an intermediate V_H vector containing a eukaryotic leader sequence derived from human antibody HG3 CL (Rechavi et al., *Proc. Natl. Acad. Sci. USA* 1983. *80:* 855) and a splice donor site (Fig. 3). The DNAs coding for the V_ks were adapted for insertion into an intermediate V_k vector using PCR primers to incorporate XhoI and SstI sites at the 5'- and 3'- ends (VkFor: 5'-CCG TTT CAG CTC GAG CTT GGT CCC-3', VkBack: 5'-GAC ATT GAG CTC ACC CAG TCT CCA-3'). The SstI-XhoI fragments were cloned into the intermediate V_k vector containing a eukaryotic leader sequence derived from reshaped

human CAMPATH-1 light chain (Riechmann et al., *Nature* 1988. *332:* 21) and a splice donor site (Fig. 3). The DNAs coding for the variable regions plus eukaryotic flanking regions were cloned as HindIII-BamHI fragments into mammalian cell expression vectors containing genomic DNAs coding for human gamma-1 constant region or human kappa constant region (Maeda et al., *Hum. Antibod. Hybridomas* 1991. *2:* 124). The heavy and light chain expression vectors were electroporated into COS cells. After 72 h, medium was collected and the chimeric anti-EGFR antibodies analyzed by ELISA (Kettleborough et al., *Protein Eng.* 1991. *4:* 773).

EXAMPLE 10: Production of sc Fvs derived from *in vitro* immunized cells.

The methods disclosed below are slight modifications of the methods described above. Immunization, library construction and screening are given in Examples 1 - 4. The following steps are described in detail below:

After screening the primary library and the clones derived from the three rounds of panning, some single ampicillin-resistant colonies were selected. Phagemid DNA was prepared by alkaline lysis and used to transfect *E.coli* HB2151, a non-supressor strain, by heat shock. Colonies were inoculated into 2xTY-Amp-Glu and grown overnight at 30°C. A 5 ml aliquot was used to inoculate 50 ml of 2xTY broth containing 100 mg ampicillin/ml and 0.1% glucose and grown with shaking at 30°C for 1 h (until log-phase). Cells were harvested and expression of soluble scFv was induced by the addition of isopropyl ß-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM (De Bellis, D. and Schwartz, I.; Nucleic Acids Res.; 1990. 18: 1311). Cultures were grown overnight at 30°C

with shaking. Supernatants containing scFv were taken, clarified by centrifugation and filtration through 0.22 mm filters and tested. Bacterial supernatants were tested for binding to EGFR by ELISA, as described (Kettleborough, et al., EP 94104160 and Eur. J. Immunol. 1994. 24: 952). The specificity of selected scFv fragments was checked by ELISA using plates coated with various proteins related and non-related to EGFR, as well as other antigens and plastic. The antigens used were: RNase, BSA, OVA, GD_3 ganglioside, vitronectin receptor (VNR), platelet glycoprotein IIbIIIa (GPIIbIIIa), and disialyl-lacto-N-tetraose (DSLNT). Coating was done overnight at the optimum concentration for each antigen. Coated ELISA plates were blocked for 1 h at 37°C with 1.5% skimmed milk in PBS (w/v). After washing, 100 ml of scFv supernatants were added to the microtiter wells and incubated for 2 h at 37°C. Bound scFv were detected using the anti-*c-myc* antibody 9E10 (spent culture media from Myc 1-9E10.2 hybrid) and an alkaline phosphatase-conjugated rabbit anti-mouse antibody (Dako).

Three EGFR-bearing tumor cell lines, A431, MDA MB 231 human breast adenocarcinoma (ATCC, HTB 26), and HT29 human colon adenocarcinoma (ATCC, HTB 38), and one non-expressing EGFR cell line, WM164, were used to test the ability of scFv to bind to EGFR on cells by mean FACS analysis and immunofluorescence with unfixed cells. For the indirect immunofluorescence analysis, cells were plated into Terasaki plates ($2x10^4$ cells/well) and cultured for 24 h. Cells were them incubated with 20 ml of crude bacterial supernatant containing the scFv fragments for 90 min at room temperature. Incubations with primary antibody (anti-c-*myc*) and secondary antibody were carried out for 60 min at room temperature. The secondary antibody, FICT-conjugated rabbit anti-mouse antibody (Dako) was diluted 1:20.

For FACS analysis, $5x10^5$ cells were washed with PBS with 1% BSA and 0.1% sodium azide (PBS-BSA) and incubated at 4°C for 20 min with 50 ml of crude bacterial supernatant. After two washes with cold PBS-BSA, bound scFv was detected using anti-c-*myc* antibody and FITC-conjugated goat anti-mouse antibody (Becton-Dickinson) diluted 1:25 in PBS-BSA. Propidium iodide (PI) was added at a final concentration of 5 mg/ml. Flow cytometry analysis were performed in a EPICS Profile II equipped with an air-cooled argon laser. The 488 nm line (15 mV) was used for the excitation. A 530 nm band pass filter was used to collect FITC emission and a 625 nm band pass filter was used to collect PI emission. Living cells were selected by setting a bitmap on forward and side scatter and by exclusion of PI-stained cells.

The diversity of the primary and selected libraries was determined by PCR amplification of cloned fragments (Gussow, D. Clackson, T; Nucleic Acids Res. 1989. 17: 4000) and analysis of the BstNI digestion pattern (8). Some clones were sequenced using a Sequenase kit (USB) by the dideoxy chain termination method (Sanger, F et al.; Proc. Nat.Acad. Sci., U.S.A. 1977. 74: 5463).

Crude bacterial supernatants (10 ml) were subjected to SDS-PAGE using a 12.5% gel. Western blotting was performed essentially as described by Towbin (Towbin et al. J. Proc.Nat.Acad.Sci., U.S.A. 1979. 76: 4350). Proteins were transferred by electroblotting to Immobilon-P (Millipore) or nitrocellulose (Bio-Rad). The blot was blocked with PBS containing 2% skimmed milk (w/v). scFv fragments were detected using anti-c-*myc* antibody (9E10), peroxidase-conjugated anti-mouse antibody (Jackson), and an enhanced chemiluminiscence system (ECL, Amersham).

The quantitative analysis of the shed membrane vesicles revealed a total protein concentration of 2.5 mg/ml, of which only 10-14% corresponded to EGFR (Sato et al.; J. Natl. Cancer Inst. 1989. 21: 1601; Yeaton, R et al.,

J. Biol. Chem., 1983. 258: 9254), 250 to 350 ng/ml. Electrophoretic analysis using PAGE-SDS followed by Coomassie-blue staining showed that the vesicles contained a rather complex mixture of proteins. No protein degradation was detected. Western blot analysis revealed that under our experimental conditions complete molecules of EGF receptor were present in the membrane vesicle preparation.

In order to determine the requirements for FCS and limphokines MTC and M2 containing 20% or 4% FCS were compared. Vesicles bearing EGFR and PBS were used as antigen and control respectively. Splenocytes were incubated in six well plates with or without antigen for 3 h in M1 (serum-free). MTC or M2 was then added and, after 72, 96, 120 or 144 h, screening was carried out using A431 fixed cells. In all experiments, the number of viable cells recovered was between 20 and 40% in agreement with published results (Gavilondo-Cowley, J. et al.; In Vitro Immunization in Hybridoma Technology, Elsevier Science Publishers B.V., Amsterdam 1988, p. 131). The maximum specific response was obtained on day four with MTC; whereas, M2 at 4% or 20% FCS (2% or 10% final concentration) delayed the maximum response until day six (Table 2). However MTC and 10% FCS triggered a non-specific response, probably by polyclonal activation, as could be seen when the results were expressed as the ratio of specific / non-specific response. For further assays we decide to use M2 supplemented with 4% FCS and 6 d of culture.

The presence of EGFR in the suface of vesicles strongly enhanced the response to this antigen. In similar protocols as described above, vesicles from expressing and non-expressing EGFR cell lines were compared. Lymphocytes were cultured with vesicles in M1 for 3 h. Afterwards M2 containing 4% FCS was added. After 6 d, lymphocytes from each group were cultured for 48 h in 96 well plates coated with EGFR, A431-fixed cells, RNase or GD3. As expected, the results of these assays showed a

multispecific pattern of response (Table 3). The reactivity against EGFR was clearly increased in terms of optical density when EGFR-expressing vesicles were used as antigen.

Taken together, these results suggest that, although immature, there was a measurable antigen-dependent response after *in vitro* immunization which generated several pools of immunized lymphocytes against EGFR suitable for PCR-cloning of variable regions.

A library of $1.1x10^5$ clones was obtained after cloning scFv fragments derived from *in vitro* immunization into the pHEN1 phagemid. This library was generated in parallel with two more libraries providing of *in vivo* immunization. The construction of these phages libraries has been described previously (Kettleborough, et al., EP 94104160 and Eur. J. Immunol. 1994. 24: 952).

To select the scFv fragments binding to EGFR, phage were panned using EGFR-coated immunotubes. Eluted phage were used to reinfect a *SupE* strain of *E.coli*. In total, three rounds of selection were carried out. In each round, a tube without antigen was tested in paralel to calculate the background. In the first panning, $1.5x10^{10}$ phage particles were applied to the immunotube and $6.6x10^4$ were eluted from the coated immunotube; whereas, only 200 colonies were obtained from the background population. After the third panning, $1x10^{11}$ phages were applied and $5.6x10^{10}$ were eluted.

To further characterize the scFv fragments, we selected 22 clones from the phage populations, before selection and after each round of selection.

The diversity of the library was analyzed by the BstNI digestion patterns of the cloned fragments. Prior to selection the library appeared to be extremely diverse. Fingerprinting of binding clones derived after the first

round of selection indicated the presence of several groups with the same restriction pattern.

Clones were selected from different rounds of selection based on their digestion patterns. DNA sequencing revealed the presence of different sequences in most of the selected clones. The length and composition of complementarity determining regions (CDRs) of clones 10D2, 5D3, 10E2, 1B3, 4B3 and 5E2 were different. The most variation was observed in the CDR3s of V_H and V_L sequences. Clones 5D3 and 1E3 were derived from the third round of selection. They bound strongly to EGFR as analyzed by ELISA and flow cytometry and had the same sequence.

Soluble scFv fragments were obtained by growth of the non-suppressor *E.coli* strain HB 2151 in presence of IPTG.

To verify scFv production, bacterial medium from individual clones, was analyzed by gel electrophoresis. Western blot analysis revealed a clear band around 35,000 kD.

Clones with binding activities to EGFR were identified by ELISA. To examine the cross-reactivity of selected clones, ELISA assays using different antigens were carried out. The antigens (EGFR, RNase, BSA, KLH, OVA, GD_3 ganglioside, vitronectin receptor, platelet glycoprotein IIbIIIa, and disialyl-lacto-N-tetraose) were coated into ELISA plates at the optimum concentration (Table 4). No binding to non-EGFR antigens was detected. The scFvs were also tested for binding to three EGFR-bearing tumor cell lines (human epidermoid carcinoma A431, human breast adenocarcionma MDA MB 231 and human colon adenocarcinoma HT 29). WM 164 a human melanoma non-expressing EGFR was used as a negative control. Those that bound to tumor cell lines was tested by indirect immunofluorescence using unfixed cells and quantified by FACS analysis. The use of unfixed cells ensures the natural conformation of the membrane receptors. Positive clones showed a clear fluorescence using

A431 cells. Fluorescence with the others EGFR-bearing tumor cell lines was weak. No binding to the negativ cell line was detected. The results were confirmed by flow cytometry. Seventeen positive clones and three negative clones were analyzed for binding to A431, MDA MB 231 and HT 29 cells by flow cytometry. WM 164 was used as the negative cell line. The 425 scFv (P1 clone) was used as a positive control and the cloning vector (HEN) as a negative control. The results are summarized in Table 5. Two clones, 4B2 and 5E2, were positive for binding to EGFR, as analyzed by ELISA, but negative for binding to EGFR-expressing tumor cell lines.

Table 2. Effect of different media on *in vitro* immunization. [a]

		Day of screening against of A431							
		3th day		4th day		5th day		6th day	
Assay	Antigen	O.D.[c]	Ratio[d]	O.D.	Ratio	O.D.	Ratio	O.D.	Ratio
1	Vesicles	0.393	2.11	0.801	3.76	0.784	3.90	0.951	10.3
	PBS	0.186		0.213		0.201		0.092	
2	Vesicles	0.527	2.50	0.852	1.76	0.863	2.75	1.168	3.94
	PBS	0.210		0.482		0.313		0.296	
3	Vesicles	0.763	1.48	1.169	2.01	1.089	2.07	1.115	1.91
	PBS	0.513		0.581		0.525		0.581	

Assay 1: M1 plus M2, 4% FCS (Final FCS: 2%)

Assay 2: M1 plus M2, 20% FCS (Final FCS: 10%)

Assay 3: A-medium plus MTC, 4% FCS (Final FCS: 2%)

a) BALB/c mouse spleen cells (10^7) were incubated in 3.5 ml of M1 with vesicles from A431 cells or PBS for 3 h in wells of 6 well plates. Afterwards 3.5 ml of MTC or M2 containing 4% or 20% FCS were added and the plates incubated. At 3, 4, 5 or 6 days *in vitro* immunized lymphocytes were removed from culture medium, washed in HBSS to remove vesicles and seeded in 96 well plates coated with fixed A431 cells, and incubated for 48 h (see Methods).

b) Final concentration of FCS in culture medium.

c) O.D. Optical densitiy read at 405nm. It represents the mean of sixteen wells.

d) Ratio of specific response (vesicles as antigen) / unspecific response (PBS as antigen).

Table 3. Multi-specificity of the response after *in vitro* immunization[a]

		Screening against			
	Antigen group	A431 CELLS	EGFR	GD3	RNase
Assay 1	EGFR +	0.512 [b]	0.326	0.140	0.249
	EGFR -	0.427	0.070	0.123	0.304
Assay 2	EGFR +	1.430	0.730	0.233	0.670
	EGFR -	0.789	0.195	0.118	0.561

a) Lymphocytes were *in vitro* immunized using either EGFR-expressing vesicles (EGFR+) or non-EGFR expressing vesicles (EGFR-). After six days of incubation, cells were removed from culture and screened against the above mentioned antigens.

b) Response is expressed as optical density (405 nm).

Table 4. Cross-reactivity of selected scFv fragments against several antigens [a]

ANTIGEN [b]	COATING [mg/ml]	RESULT
EGFR	2.5	+
RNase	10	-
BSA	10	-
KLH	10	-
OVA	10	-
GD_3 ganglioside	2	-
VNR	1	-
GPIIbIIIa	1	-
DSLNT	5	-

a) ELISA assays were performed as described.

b) Vitronectin receptor (VNR) ; platelet glycoprotein IIbIIIa (GPIIbIIIa); disialyl-lacto-N-tetraose (DSLNT).

Table 5. Reactivity of scFv clones against EGFR. Comparative results between an ELISA method with purified soluble antigen and cytometric analysis of cell lines.

CLONES	CYTOMETRIC ANALYSIS OF TUMOR CELL LINES [a] (mean of arbritary fluorescence units)				ELISA (O.D.)
Postive	WM164	A431	MDAAMB231	HT29	EGFR
7H1	1.5	112.9	16.4	2.6	1.2
4B2	1.2	5.3	4.2	0.6	2
10D2	1.5	145.3	36.3	4.8	2
12D2	1.8	129.5	29.3	5.7	2
5E2	1.4	2.5	7.1	0.5	1.8
8E2	1.5	134.5	47.7	5.1	1.9
5F2	1.3	146.3	40.6	5.7	1.9
11H2	1.9	152.2	25.3	2	1.9
1B3	0.6	105.1	36.4	5.2	>2
4B3	0.5	78	15.8	2.3	2
3D3	1.2	94.3	25.1	4.8	1.9
5D3	0.5	112	22.2	5.5	>2
4F3	0.4	110.3	32.3	6.2	>2
4G3	0.4	76.5	20.4	2	>2
1E3	0.4	118.3	33.8	5.1	2
3H3	0.6	76.5	33.7	4.2	>2
Negative					
5F1	2.4	2.3	3.6	1.8	0.2
7G1	1.4	10.2	4	2.8	0.2
1H1	0.5	5	4	0.75	0.2

Table 5: continuation

Controls [b]					
HEN	0.4	4.1	3.7	1	0.2
P1	0.6	85.5	21.3	2.5	1.9

a) Three EGFR-bearing cell lines (A431, MDAAMB231 and HT29) and one non-expressing cell line (WM164) were used to assay the ability of scFv to bind to tumor cells lines by cytometric analysis as described.

b) Vector without fragment (HEN) and scFv fragment from 425 mAb (P1) were used as negative and positive controls, respectively.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Merck Patent GmbH
(B) STREET: Frankfurter Str. 255
(C) CITY: Darmstadt
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 64271
(G) TELEPHONE: 49-6151-727022
(H) TELEFAX: 49-6151-727191

(ii) TITLE OF INVENTION: Anti-EGFR Single-Chain Fvs and Anti-EGFR Antibodies

(iii) NUMBER OF SEQUENCES: 32

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:
(B) CLONE: L2 11C (light chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..327

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GAC ATT GAG CTC ACC CAG TCT CCA GCC TCC CTG GCT GCA TCT GTG GGA    48
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Val Gly
 1               5                  10                  15
```

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GAA ACT GTC ACC ATC ACA TGT CGA GCA AGT GAG AAC ATT TAC TAT AGT     96
Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Tyr Ser
             20                  25                  30

TTA GCA TGG TAT CAG CAG AAG CAA GGG AAA TCT CCT CAG CTC CTG ATC    144
Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile
         35                  40                  45

TAT AGT GCA AGC GCC TTG GAA GAT GGT GTC CCA TCG AGG TTC AGT GGC    192
Tyr Ser Ala Ser Ala Leu Glu Asp Gly Val Pro Ser Arg Phe Ser Gly
     50                  55                  60

AGT GGA TCT GGG ACA CAG TAT TCT TTA AAG ATC AAC AAC ATG CAG CCT    240
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Asn Met Gln Pro
 65                  70                  75                  80

GAA GAT ACC GCT ACT TAC TTC TGT AAA CAG ACT TAT GAC GTT CCG TGG    288
Glu Asp Thr Ala Thr Tyr Phe Cys Lys Gln Thr Tyr Asp Val Pro Trp
                 85                  90                  95

ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA CGG GCG                327
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
            100                 105
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Val Gly
 1               5                  10                  15

Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Tyr Ser
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Ala Leu Glu Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Asn Met Gln Pro
65                  70                  75                  80

Glu Asp Thr Ala Thr Tyr Phe Cys Lys Gln Thr Tyr Asp Val Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
           100                 105
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 357 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph Node

(vii) IMMEDIATE SOURCE:
(B) CLONE: L2 11C (heavy chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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CAG GTG CAA CTG CAG GAG TCA GGG CCT GAG CTG GTG AGG CCT GGG GCT    48
Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Arg Pro Gly Ala
110                 115                 120                 125

TCA GTG AAG ATG TCC TGC AAG GCT TCA GGC TAT ACC TTC ACT ACC TAC    96
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr
                130                 135                 140

TGG ATA CAC TGG ATG AAA CAG AGG CCT GGA CAA GGC CTT CAG TGG ATT   144
Trp Ile His Trp Met Lys Gln Arg Pro Gly Gln Gly Leu Gln Trp Ile
            145                 150                 155

GGC ATG ATT GAT CCT TCC AAT AGT GAA ACT AGG TTA AAT CAG AAT TTC   192
Gly Met Ile Asp Pro Ser Asn Ser Glu Thr Arg Leu Asn Gln Asn Phe
        160                 165                 170

AGG GAC AAG GCC ACA TTG AGT GTA GAC AAA TCC TCC AAT AAA GCC TAC   240
Arg Asp Lys Ala Thr Leu Ser Val Asp Lys Ser Ser Asn Lys Ala Tyr
    175                 180                 185

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCA ATC TAT TAC TGT   288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
190                 195                 200                 205

GCA AGA TGG GAC TAC GGT AGT GGC CAC TTT GAC TAC TGG GGC CAA GGG   336
Ala Arg Trp Asp Tyr Gly Ser Gly His Phe Asp Tyr Trp Gly Gln Gly
                210                 215                 220

ACC ACG GTC ACC GTC TCC TCA                                      357
Thr Thr Val Thr Val Ser Ser
            225
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Arg Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr
            20                  25                  30

Trp Ile His Trp Met Lys Gln Arg Pro Gly Gln Gly Leu Gln Trp Ile
        35                  40                  45

Gly Met Ile Asp Pro Ser Asn Ser Glu Thr Arg Leu Asn Gln Asn Phe
    50                  55                  60

Arg Asp Lys Ala Thr Leu Ser Val Asp Lys Ser Ser Asn Lys Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Trp Asp Tyr Gly Ser Gly His Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:
(B) CLONE: L2 12B (light chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG     48
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
120                 125                 130                 135

CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC GAA AGT GTT GAT AAT TTT     96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
                140                 145                 150
```

```
GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC   144
Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
            155                 160                 165

AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC   192
Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
        170                 175                 180

AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT   240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
    185                 190                 195

CCT CTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG   288
Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
200                 205                 210                 215

GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA ATA AAA CGG   336
Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
                220                 225                 230

GCG                                                              339
Ala
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
            20                  25                  30

Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
                85                  90                  95

Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105                 110

Ala
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 357 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:
(B) CLONE: L2 12B (heavy chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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CAG GTG CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGG GCT      48
Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
115                 120                 125

TTA GTG AAG ATA TCC TGC AAG GCT TCT GGT TAC ACC TTC ACC AGC TAC      96
Leu Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
130                 135                 140                 145

TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATC     144
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
                    150                 155                 160

GGA GAG ATT GAT CCT TCT GAT AGT TAT ACT AAC TAC AAT CAA AAG TTC     192
Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
            165                 170                 175

AAG GGC AAG GCC ACA TTG ACT GTA GAC AAA TCC TCC AAC ACA GCC TAC     240
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
        180                 185                 190

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT     288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
    195                 200                 205

GCA AGA TCG GAC TAC GGT AGT AGC CAC TTT GAC TAC TGG GGC CAA GGG     336
Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
210                 215                 220                 225

ACC ACG GTC ACC GTC TCC TCA                                        357
Thr Thr Val Thr Val Ser Ser
                230
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15

Leu Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:
(B) CLONE: L3 11D (light chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG     48
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
120                 125                 130                 135

CAG AGG GCC ACC ATC TCC TGC CGA GCC AGC GAA AGT GTT GAT AAT TTT     96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
                140                 145                 150
```

```
GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC   144
Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
            155                 160                 165

AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC   192
Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
        170                 175                 180

AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT   240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
    185                 190                 195

CCT TTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG   288
Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
200                 205                 210                 215

GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGG   336
Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
                220                 225                 230

GCG                                                              339
Ala
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
            20                  25                  30

Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
                85                  90                  95

Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
            100                 105                 110

Ala
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 357 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:
(B) CLONE: L3 11D (heavy chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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GAG GTG CAG CTG CAG CAG TCA GGG GCT GAG CTT GTG AAG CCT GGG GCT      48
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
    115                 120                 125

TCA GTG AAG CTG TCC TGC AAG GCT TCT GGC TAC ACC TTC ACC AGC TAC      96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
130                 135                 140                 145

TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATC     144
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
                150                 155                 160

GGA GAG ATT GAT CCT TCT GAT AGT TAT ACT AAC TAC AAT CAA AAG TTC     192
Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
            165                 170                 175

AAG GGC AAG GCC ACA TTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC     240
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
        180                 185                 190

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT     288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
    195                 200                 205

GCA AGA TCG GAC TAC GGT AGT AGC CAC TTT GAC TAC TGG GGC CAA GGG     336
Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
210                 215                 220                 225

ACC ACG GTC ACC GTC TCC TCA                                        357
Thr Thr Val Thr Val Ser Ser
                230
```

(2) INFORMATI N FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Val Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:
(B) CLONE: S4 2D (light chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..327

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
GAC ATT GAG CTC ACC CAG TCT CCA ACC ACC ATG GCT GCA TCT CCC GGG     48
Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Met Ala Ala Ser Pro Gly
120                 125                 130                 135

GAG AAG ATC ACT ATC ACC TGC AGT GCC AGC TCA AGT ATA AGT TCC AAT     96
Glu Lys Ile Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Ser Asn
                140                 145                 150

TAC TTG CAT TGG TAT CAG CAG AAG CCA GGA TTC TCC CCT AAA CTC TTG    144
Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Phe Ser Pro Lys Leu Leu
            155                 160                 165

ATT TAT AGG ACA TCC AAT CTG GCT TCT GGA GTC CCA GCT CGC TTC AGT    192
Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
        170                 175                 180

GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATT GGC ACC ATG GAG    240
Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Gly Thr Met Glu
    185                 190                 195

GCT GAA GAT GTT GCC ACT TAC TAC TGC CAG CAG GGT AGT AGT ATA CCA    288
Ala Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ser Ile Pro
200                 205                 210                 215

CGC ACG TTC GGA GGG GGC ACC AAG CTG GAA ATC AAA CGG                327
Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg.
                220                 225
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Met Ala Ala Ser Pro Gly
 1               5                  10                  15

Glu Lys Ile Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Ser Asn
            20                  25                  30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Phe Ser Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
    50                  55                  60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Gly Thr Met Glu
65                  70                  75                  80

Ala Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ser Ile Pro
                85                  90                  95

Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:
(B) CLONE: S4 2D (heavy chain)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
GAG GTC AAG CTG CAG CAG TCA GGA CCT GAG CTG GTA AAG CCT GGG GCT     48
Glu Val Lys Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
110                 115                 120                 125

TCA GTG AAG ATG TCC TGC AAG GCT TCT GGA TAC GCA TTC ATA AGT TTT     96
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ile Ser Phe
                130                 135                 140

GTT ATG CAC TGG GTG AAG CAG AAG CCT GGG CAG GGC CTT GAG TGG ATT    144
Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
            145                 150                 155

GGA TTT ATT AAT CCT TAC AAT GAT GGT ACT AAG TAC AAT GAG AAG TTC    192
Gly Phe Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
        160                 165                 170

AAA GAC AAG GCC ACA CTG ACT TCA GAC AAA TCC TCC AGC ACA GCC TAC    240
Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
    175                 180                 185

ATG GAG CTC AGC AGC CTG ACC TCT GAG GAC TCT GCG GTC TAT TAC TGT    288
Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
190                 195                 200                 205

GCA AGT GGG GAT TAC GAC AGG GCT ATG GAC TAC TGG GGC CAA GGG ACC    336
Ala Ser Gly Asp Tyr Asp Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr
                210                 215                 220

ACG GTC ACC GTC TCC TCA                                            354
Thr Val Thr Val Ser Ser
            225
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Glu Val Lys Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ile Ser Phe
            20                  25                  30

Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Phe Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Gly Asp Tyr Asp Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 717 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(B) CLONE: 4 B 2

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..717

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
GAG GTG AAG CTG CAG GAG TCT GGG GGA GAC TTA GTG AAG CCT GGA GGG     48
Glu Val Lys Leu Gln Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
    120                 125                 130

TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AGC TAT     96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
135                 140                 145                 150

GGC ATG TCT TGG GTT CGG CAG ACT CCA GAC AAG AGG CTG GAG TCT GTC    144
Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Ser Val
                155                 160                 165

GCA ACC ATT AGT AGT GGT GGT GCT TAC ATC TAC TAT CCA GAC AGT GTG    192
Ala Thr Ile Ser Ser Gly Gly Ala Tyr Ile Tyr Tyr Pro Asp Ser Val
            170                 175                 180

AAG GGG CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC    240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
        185                 190                 195

CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC ATG TAT TAC TGT    288
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
    200                 205                 210

GCA AGA CTT GAA ACC GGG GAC TAT GCT TTG GAC TAC TGG GGC CAA GGG    336
Ala Arg Leu Glu Thr Gly Asp Tyr Ala Leu Asp Tyr Trp Gly Gln Gly
215                 220                 225                 230

ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT GGT GGG    384
Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
                235                 240                 245

TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT    432
Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser
            250                 255                 260

TTG GCT GTC TCT CTA GGG CAG AGG GCC ACC ATA TTC TGC AAG GAC AGC    480
Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Phe Cys Lys Asp Ser
        265                 270                 275

CAA AGT GTT GAT TAT GAT GGT GAT AGT TAT ATG AAC TGG TAC CAA CAG    528
Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln
    280                 285                 290

AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT GCT CGA TCC AAT CTA    576
Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Arg Ser Asn Leu
295                 300                 305                 310

GAA TCT GGG GTC CCT GCC AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC    624
Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
                315                 320                 325

TTC AGC CTC AAC ATC CAT CCT GTG GAG GAG GAT GAT ATT GCA ATG TAT    672
Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr
            330                 335                 340

TTC TGT CAG CAA AGT AGG AAG GTT CCG TGG TCG TTC GGT GGA GGG        717
Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Ser Phe Gly Gly Gly
        345                 350                 355
```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Glu Val Lys Leu Gln Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Ser Val
35 40 45

Ala Thr Ile Ser Ser Gly Gly Ala Tyr Ile Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Leu Glu Thr Gly Asp Tyr Ala Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser
130 135 140

Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Phe Cys Lys Asp Ser
145 150 155 160

Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln
165 170 175

Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Arg Ser Asn Leu
180 185 190

Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
195 200 205

Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr
210 215 220

Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Ser Phe Gly Gly Gly
225 230 235

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 732 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(B) CLONE: 10 D 2 (single-chain Fv, heavy and light chain plus linker)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
GAG GTG CAG CTG CAG CAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT     48
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
240                 245                 250                 255

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC     96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
                260                 265                 270

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC    144
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
            275                 280                 285

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC    192
Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
        290                 295                 300

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC    240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
    305                 310                 315

ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT    288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
320                 325                 330                 335

GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC    336
Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
                340                 345                 350

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT    384
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
            355                 360                 365

GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA    432
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
        370                 375                 380

GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT    480
Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
    385                 390                 395

GCC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAA CCA GGA    528
Ala Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Pro Gly
400                 405                 410                 415
```

```
TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA   576
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
                420                 425                 430

GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC   624
Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
            435                 440                 445

ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG   672
Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
        450                 455                 460

CAG TGG AGT AGT TAC CCA CCC ATG TAC ACG TTC GGA GGG GGG ACC AAG   720
Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
    465                 470                 475

CTG GAA ATA AAA                                                  732
Leu Glu Ile Lys
480
```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 244 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
  1               5                  10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
        115                 120                 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
    130                 135                 140

Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
145                 150                 155                 160

Ala Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Pro Gly
                165                 170                 175
```

```
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
            180                 185                 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
        195                 200                 205

Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
    210                 215                 220

Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
225                 230                 235                 240

Leu Glu Ile Lys
```

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 732 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(B) CLONE: 3 D 3 (single-chain Fv, heavy and light chain plus linker)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
GAG GTC CAA CTG CAG CAG TCA GGG GCT GAA CTG GTG AAG CCT GGG GCT     48
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
245                 250                 255                 260

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC     96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
                265                 270                 275

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC    144
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
            280                 285                 290

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA ATC    192
Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Ile
        295                 300                 305

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC    240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
    310                 315                 320
```

```
ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT   288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
325                 330                 335                 340

GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC   336
Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
                345                 350                 355

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT   384
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
            360                 365                 370

GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA   432
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
        375                 380                 385

ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT   480
Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
    390                 395                 400

GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG ACA GGA   528
Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
405                 410                 415                 420

TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA   576
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
                425                 430                 435

GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC   624
Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
            440                 445                 450

ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG   672
Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
        455                 460                 465

CAG TGG AGT AGT TAC CCA CCC ATG TAC ACG TTC GGA GGG GGG ACC AAG   720
Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
    470                 475                 480

CTG GAA ATA AAA                                                  732
Leu Glu Ile Lys
485
```

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 244 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
  1               5                  10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45
```

Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Ile
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
130 135 140

Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
145 150 155 160

Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
165 170 175

Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
180 185 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
195 200 205

Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
210 215 220

Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
225 230 235 240

Leu Glu Ile Lys

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 738 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(B) CLONE: 1 E 3 (single-chain Fv, heavy and light chain plus linker)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..738

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
GAG GTG CAG CTG CAG CAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT     48
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
245                 250                 255                 260

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC     96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
                265                 270                 275

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC    144
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
            280                 285                 290

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC    192
Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
        295                 300                 305

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCT TAC    240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
    310                 315                 320

ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT    288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
325                 330                 335                 340

GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC    336
Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
                345                 350                 355

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT    384
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
            360                 365                 370

GGT GGG TCG GGT GGC GGC GGA TCT GGA TCT GAC ATT GAG CTC ACC CAG    432
Gly Gly Ser Gly Gly Gly Gly Ser Gly Ser Asp Ile Glu Leu Thr Gln
        375                 380                 385

TCT CCA ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC    480
Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr
    390                 395                 400

TGC AGT GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG    528
Cys Ser Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys
405                 410                 415                 420

CCA GGA TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT    576
Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala
                425                 430                 435

TCT GGA GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC    624
Ser Gly Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr
            440                 445                 450

TCT CTC ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC    672
Ser Leu Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr
        455                 460                 465

TGC CAG CAG TGG AGT AGT TAC CCA CCC ATG TAC ACG TTC GGA GGG GGG    720
Cys Gln Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly
    470                 475                 480
```

```
ACC AAG CTG GAA ATA AAA                                                738
Thr Lys Leu Glu Ile Lys
485                 490
```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 246 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
        115                 120                 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Ser Asp Ile Glu Leu Thr Gln
    130                 135                 140

Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr
145                 150                 155                 160

Cys Ser Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys
                165                 170                 175

Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala
            180                 185                 190

Ser Gly Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr
        195                 200                 205

Ser Leu Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr
    210                 215                 220

Cys Gln Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly
225                 230                 235                 240

Thr Lys Leu Glu Ile Lys
                245
```

(2) INFORMATI N FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 726 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(B) CLONE: 5 F 1 (single-chain Fv, heavy, light chain, linker)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..726

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
CAG GTG AAA CTG CAG GAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT     48
Gln Val Lys Leu Gln Glu Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
            250                 255                 260

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC     96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
        265                 270                 275

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC    144
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
    280                 285                 290

GGA GAG ATT AAT CCC AGA ACG GCG CCT ACT AAC TAC AAT GAG AAA TTC    192
Gly Glu Ile Asn Pro Arg Thr Ala Pro Thr Asn Tyr Asn Glu Lys Phe
295                 300                 305                 310

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC    240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
                315                 320                 325

ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT    288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
            330                 335                 340

GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC    336
Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
        345                 350                 355

CAA GGG ACA ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT    384
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
    360                 365                 370
```

```
GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA   432
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
375                 380                 385                 390

ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT   480
Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
                395                 400                 405

GAC AGC TCA AGT GTA AGT TAC ACG TAC TGG TAC CAG CAG AAG ACA GGA   528
Asp Ser Ser Ser Val Ser Tyr Thr Tyr Trp Tyr Gln Gln Lys Thr Gly
            410                 415                 420

TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA   576
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
        425                 430                 435

GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC   624
Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
    440                 445                 450

ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG   672
Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
455                 460                 465                 470

CAG TGG AGT AGT TAC CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA   720
Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu
                475                 480                 485

ATA AAA
Ile Lys                                                         726
```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```
Gln Val Lys Leu Gln Glu Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Asn Pro Arg Thr Ala Pro Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
            100                 105                 110
```

```
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
        115                 120                 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
    130                 135                 140

Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
145                 150                 155                 160

Asp Ser Ser Ser Val Ser Tyr Thr Tyr Trp Tyr Gln Gln Lys Thr Gly
                165                 170                 175

Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
            180                 185                 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
        195                 200                 205

Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
    210                 215                 220

Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu
225                 230                 235                 240

Ile Lys
```

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 726 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: 7 G 1 (single-chain Fv, heavy, light chain, linker)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..726

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
GAG GTC AAG CTG CAG CAG TCA GGG GCT GAA CTG GTG AAG CCT GGG GCT    48
Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
    245                 250                 255

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC    96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
260                 265                 270
```

```
TTG GAT CAC TGG GTG AAG CAG AGG GGC TGG CAA GGC CTT GAG TGG ATC   144
Leu Asp His Trp Val Lys Gln Arg Gly Trp Gln Gly Leu Glu Trp Ile
275                 280                 285                 290

GGA CAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC   192
Gly Gln Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
                295                 300                 305

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC   240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
            310                 315                 320

ATC GAA CTC AGC AGC CTG ACA TCT GAG GAC TGC TCG GTC TAT TAC TGT   288
Ile Glu Leu Ser Ser Leu Thr Ser Glu Asp Cys Ser Val Tyr Tyr Cys
        325                 330                 335

GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC   336
Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
    340                 345                 350

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT   384
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
355                 360                 365                 370

GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA   432
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
                375                 380                 385

ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT   480
Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
            390                 395                 400

GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG ACA GGA   528
Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
        405                 410                 415

TCC TCC CCC AGA CTT CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA   576
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
    420                 425                 430

GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC   624
Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
435                 440                 445                 450

ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG   672
Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
                455                 460                 465

CAG TGG AGT AGT TAC CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA   720
Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu
            470                 475                 480

ATA AAA                                                          726
Ile Lys
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
20 25 30

Leu Asp His Trp Val Lys Gln Arg Gly Trp Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gln Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Ile Glu Leu Ser Ser Leu Thr Ser Glu Asp Cys Ser Val Tyr Tyr Cys
85 90 95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
130 135 140

Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
145 150 155 160

Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
165 170 175

Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
180 185 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
195 200 205

Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
210 215 220

Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 726 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(B) CLONE: 11 H 1 (single-chain Fv, heavy and light chain plus linker

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..726

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

```
GAG GTC AAG CTG CAG CAG TCA GGG GCT GAA CTG GTG AAG CCT GGG GCT      48
Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
        245                 250                 255

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC      96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
    260                 265                 270

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC TTG GAG TGG ATC     144
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
275                 280                 285                 290

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC     192
Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
                295                 300                 305

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC     240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
            310                 315                 320

ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT     288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
        325                 330                 335

GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC     336
Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
    340                 345                 350

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT     384
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
355                 360                 365                 370

GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA     432
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
                375                 380                 385

TCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT     480
Ser Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
            390                 395                 400

GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG ACA GGA     528
Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
        405                 410                 415
```

```
TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA   576
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
    420                 425                 430

GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC   624
Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
435                 440                 445                 450

ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG   672
Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
                455                 460                 465

CAG TGG AGT AGT TAC CCA CAC ACG TTC GGT GCT GGG ACC AAG CTG GAA   720
Gln Trp Ser Ser Tyr Pro His Thr Phe Gly Ala Gly Thr Lys Leu Glu
            470                 475                 480

ATA AAA                                                          726
Ile Lys
```

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```
Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
        115                 120                 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
    130                 135                 140

Ser Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
145                 150                 155                 160

Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
                165                 170                 175
```

```
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
            180                 185                 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
        195                 200                 205

Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
    210                 215                 220

Gln Trp Ser Ser Tyr Pro His Thr Phe Gly Ala Gly Thr Lys Leu Glu
225                 230                 235                 240

Ile Lys
```

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 732 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:
(B) CLONE: 1 A 1 (single-chain Fv, heavy and light chain plus linker)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
GAG GTG CAG CTG CAG CAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT     48
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
    245                 250                 255

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC     96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
260                 265                 270

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC    144
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
275                 280                 285                 290

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC    192
Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
            295                 300                 305

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCT TAC    240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
        310                 315                 320
```

```
ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT   288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
        325                 330                 335

GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC   336
Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
    340                 345                 350

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT   384
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
355                 360                 365                 370

GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA   432
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
                375                 380                 385

ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT   480
Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
            390                 395                 400

GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG ACA GGA   528
Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
        405                 410                 415

TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA   576
Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
    420                 425                 430

GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC   624
Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
435                 440                 445                 450

ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG   672
Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
                455                 460                 465

CAG TGG AGT AGT TAC CCA CCC ATG TAC ACG TTC GGA GGG GGG ACA AAG   720
Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
            470                 475                 480

TTG GAA ATA AAA                                                  732
Leu Glu Ile Lys
        485
```

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 244 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

```
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
  1               5                  10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
            20                  25                  30

Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45
```

Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
130 135 140

Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
145 150 155 160

Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
165 170 175

Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
180 185 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
195 200 205

Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
210 215 220

Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
225 230 235 240

Leu Glu Ile Lys

Patent Claims

1. Anti-EGFR single-chain Fvs obtainable from phage-antibody libraries constructed from cells from an immunized mammalian.

2. Antibody fragment according to Claim 1 obtainable from cells of an immunized mouse.

3. Antibody fragment according to Claims 1 or 2 obtainable from cells of

(i) the lymph node,

(ii) the spleen, or

(iii) in vitro immunized cells.

4. Antibody fragment according to Claims 1 - 3, wherein the variable regions of the heavy and light chain comprise a DNA and/or an amino acid sequence selected from one of the heavy and light chain sequences given in Sequence Id. Nos. 1- 32.

5. Anti-EGFR antibody constructed from DNA sequences deriving from antibody fragments according to Claims 1 - 4 and from DNA sequences deriving from constant regions of human immunogobulins.

6. Antibody according to Claim 5 wherein the heavy constant chain region comprises the amino acid sequence of a human gamma-1 chain, and the light constant chain region comprises the amino acid sequence of a human kappa chain.

7. Process for the preparation of an anti-EGFR single-chain Fv according to one of the Claims 1 - 4 comprising the following steps:

(i) isolating RNA from immunized mammalian cells, preferably mouse cells,

(ii) synthesizing first-strand cDNA,

(iii) amplifying the V_H and V_K genes in cDNAs from the immunized cells,

(iv) cloning said genes together with suitable restriction sites into a phagemid vector,

(v) transforming prokaryotic cells with the ligation mixtures,

(vi) screening the phage libraries for phage antibodies directed to EGFR using purified EGFR, and

(vii) producing the desired single-chain Fv in prokaryotic host cells, preferably E. coli.

8. Process for the preparation of a whole anti-EGFR antibody by cloning the DNA coding for the variable regions of anti-EGFR antibody fragments produced according to Claim 7 into at least one eukaryotic expression vector containing genomic DNA which codes for the constant regions of human immunoglobulins, transforming eukaryotic cells with said vector(s) and expressing and isolating the antibody.

9. Pharmaceutical composition comprising an anti-EGFR antibody fragment according to one of the Claims 1 - 4 or a whole anti-EGFR antibody according to Claims 5 or 6.

10. Use of an anti-EGFR antibody fragment according to one of the Claims 1 - 4, or a whole anti-EGFR antibody according to one of the Claims 5 or 6 for the manufacture of a drug directed to tumors or for the diagnostic location and assessment of tumor growth.

FIGURE 1 (A)

VH:

scFv	FR 1	CDR 1	FR 2
L3 11D	EVQLQQSGAELVKPGASVKLSCKASGYTFT	SYWMH	WVKQRPGQGLEWIG
L2 12B	QVQLQESGPELVKPGALVKISCKASGYTFT	SYWMH	WVKQRPGQGLEWIG
L3 10A	QVKLQESGGDLVKPGGSLKLSCAASGFTFS	SYGMS	WVRQTPDKRLESVA
L2 1C	EVKLQQSGAELVRPEASVKLSCKTSGYIFT	NYWIH	WVKQRSGQGLEWIA
L2 8C	QVQLQESGAELVRPGASVKLSCKTSGYIFT	NYWIH	WVKQRSGQGLEWIA
L2 11C	QVQLQESGPELVRPGASVKMSCKASGYTFT	TYWIH	WMKQRPGQGLQWIG

scFv	CDR 2	FR 3
L3 11D	EIDPSDSYTNYNQKFKG	KATLTVDKSSSTAYMQLSSLTSEDSAVYYCAR
L2 12B	EIDPSDSYTNYNQKFKG	KATLTVDKSSNTAYMQLSSLTSEDSAVYYCAR
L3 10A	TISSGGAYIYYPDSVKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR
L2 1C	RIYPGNGSTYYNEKFKG	KATLTADKSSSTAYMQLSSLKSEDSAVYFCAR
L2 8C	KDLSWNGSYYNEKFKG	KATLTADKSSSTAYMQLSSLKSEDSAVYFCAR
L2 11C	MIDPSNSETRLNQNFRD	KATLSVDKSSNKAYMQLSSLTSEDSAIYYCAR

scFv	CDR 3	FR 4
L3 11D	SDYGSSHFDY	WGQGTTVTVSS
L2 12B	SDYGSSHFDY	WGQGTTVTVSS
L3 10A	LETGDYALDY	WGQGTTVTVSS
L2 1C	STSDSSLPYWYFDV	WGQGTTVTVSS
L2 8C	STSDSSLPYWYFDV	WGQGTTVTVSS
L2 11C	WDYGSGHFDY	WGQGTTVTVSS

VK:

scFv	FR 1	CDR 1	FR 2
L3 11D	DIELTQSPASLAVSLGQRATISC	RASESVDNFGISFMN	WFQQKPGQPPKLLIY
L2 12B	DIELTQSPASLAVSLGQRATISC	RASESVDNFGISFMN	WFQQKPGQPPKLLIY
L3 10A	DIELTQSPASLAVSLGQRATISC	RASESVEYYGTSLMQ	WFQQKPGQPPKLLIY
L2 1C	DIELTQSPTILSTSPGEKVTVTC	RATLGVSYMH	WYQQKPGSSPKPWIY
L2 8C	DIELTQSPAIMSASPGEKVTITC	SASSSVSYMH	WFQQKPGTSPKLWIY
L2 11C	DIELTQSPASLAASVGETVTITC	RASENIYYSLA	WYQQKQGKSPQLLIY

scFv	CDR 2	FR 3
L3 11D	GASNQGS	GVPARFSGSGSGTDFSLNIHPLEEDDTAMYFC
L2 12B	GASNQGS	GVPARFSGSGSGTDFSLNIHPLEEDDTAMYFC
L3 10A	AASNVES	GVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC
L2 1C	ATSNLAS	GVPARFSGSGSGTSYSLTISRVEAEDAATYYC
L2 8C	STSNLAS	GVPARFSGSGSGTSYSLTISRMEAEDAATYYC
L2 11C	SASALED	GVPSRFSGSGSGTQYSLKINNMQPEDTATYFC

scFv	CDR 3	FR 4
L3 11D	QQSKEVPLT	FGAGTKLELKRA
L2 12B	QQSKEVPLT	FGAGTKLEIKRA
L3 10A	QQSRKVPWT	FGGGTKLEIKRA
L2 1C	QQWISNPPT	FGGGTKLEIKRA
L2 8C	QQRNSYPHT	FGAGTKLELKRA
L2 11C	KQTYDVPWT	FGGGTKLEIKRA

FIGURE 1 (B)

VH:

scFv	FR 1	CDR 1	FR 2
S4 2D	EVKLQQSGPELVKPGASVKMSCKASGYAFI	SFVMH	WVKQKPGQGLEWIG
S4 10H	EVKLQESGGDLVKPGGSLKLSCAASGFTFS	SYGMS	WVRQTPDKRLESVA
S4 5A	EVKLQESGGDSVKPGGSLKLSCAASGFTFS	SYGMS	WVRQTPDKRLESVA
S3 12D	EVKLQQSGAELVKPGASVKLSCTASGFNIK	DTYMH	WVKQRPEQGLEWIG

scFv	CDR 2	FR 3
S4 2D	FINPYNDGTKYNEKFKD	KATLTSDKSSSTAYMELSSLTSEDSAVYYCAS
S4 10H	TISSGGAYIYYPDSVKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR
S4 5A	TISSGGAYIYYPDSVKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR
S3 12D	RIDPANGNTKYDPKFQD	RASITADTSSNTAYLQLSSLTSEDTAVYYCAS

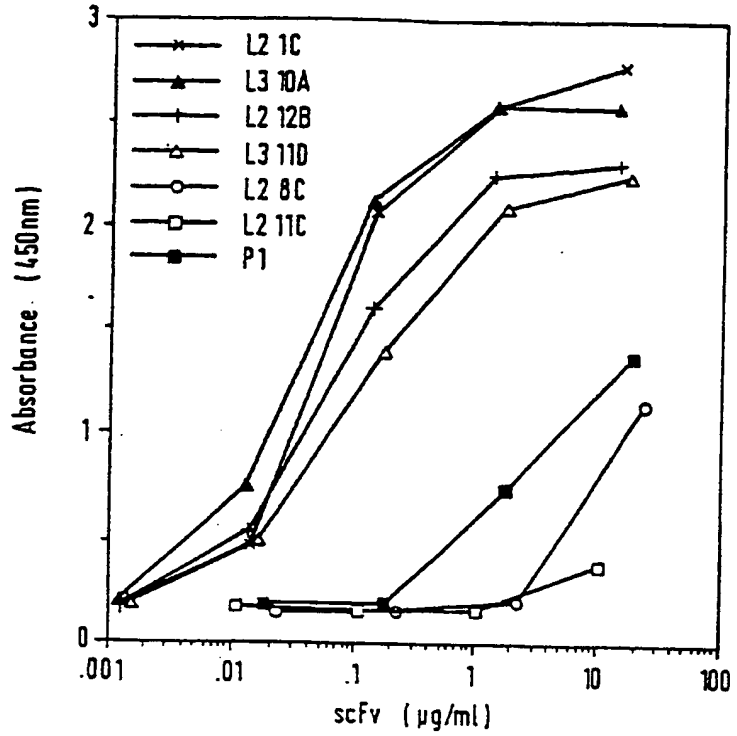
scFv	CDR 3	FR 4
S4 2D	GDYDRAMDY	WGQGTTVTVSS
S4 10H	LETGDYALDY	WGQGTTVTVSS
S4 5A	LETGDYAMDY	WGQGTTVTVSS
S3 12D	DYYGYEAWFAY	WGQGTTVTVSS

VK:

scFv	FR 1	CDR 1	FR 2
S4 2D	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY
S4 10H	DIELTQSPASLAVSLGQRATISC	RASESVEYYGTSLMQ	WYQQKPGQAPKLLIY
S4 5A	DIELTQSPASLAVSLGQRATISC	RASESVEYYGTSLMQ	WYQQKPGQPPKLLIY
S3 12D	DIELTQSPASLAVSLGQRATISC	RASESVDNYGISFMN	WYQQKPGQPPKLLIY

scFv	CDR 2	FR 3
S4 2D	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC
S4 10H	AASNVES	EVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC
S4 5A	AASNVES	GVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC
S3 12D	AASNQGS	GVPARFSGSGSGTDFSLNIHPMEEDDTAMYFC

scFv	CDR 3	FR 4
S4 2D	QQGSSIPRT	FGGGTKLEIKRA
S4 10H	QQSRKVPWT	FGGGTKLEIKRA
S4 5A	QQSRKVPWT	FGGGTKLEIKRA
S3 12D	QQSKEVPWT	FGGGTKLEIKRA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L3 10A
L2 12B
L3 11D
L2 8C
L2 11C
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Absorbance (450nm)
scFv (µg/ml)
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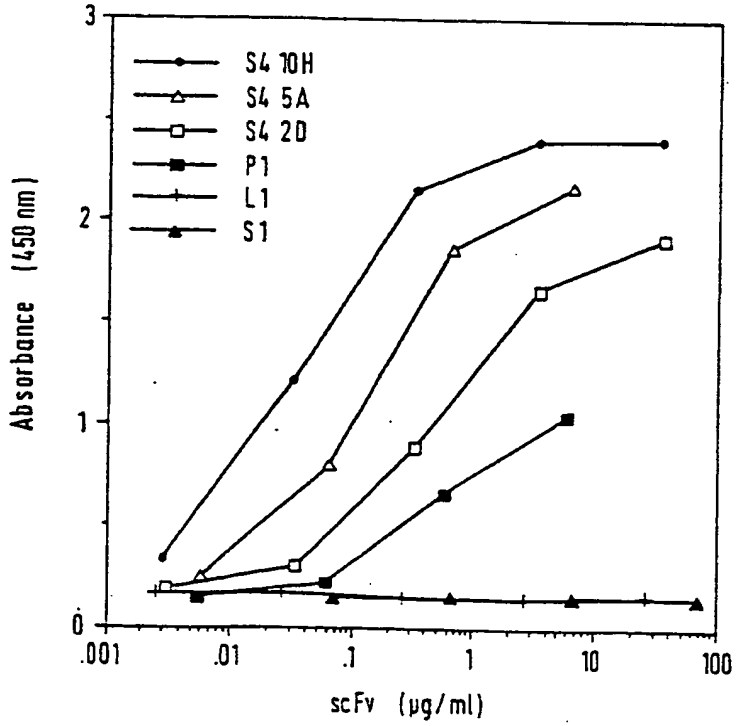
2
3
.001
.01
.1
1
10
100

Fig.2

A)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L1
S1
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scFv (µg/ml)
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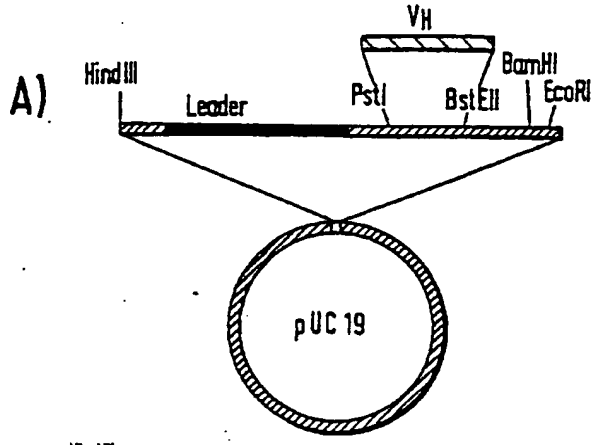
1
2
3
.001
.01
.1
1
10
100

B)

Fig.3



HindIII Leader
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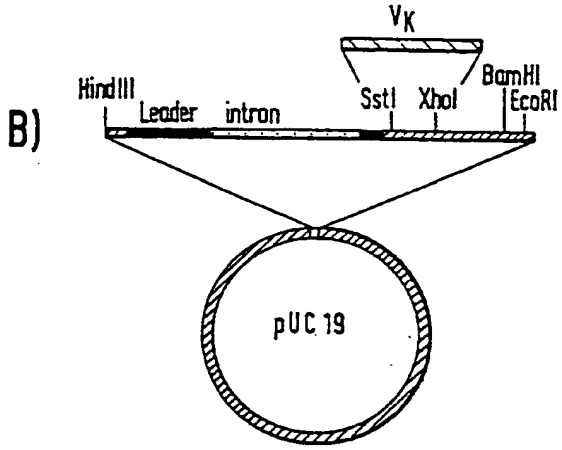
M D W T W R V F C L L A V A F
PstI BstEII
GGGCCCACAGCCAGGTGCAACTGCAGCAGTCCGGTGCCAAGGGACCACGGTCACCGTCTCC
G A H S Q V Q L Q V T V S
BamHI EcoRI
TCAGGTGAGTGGATCCGAATTC
S



HindIII Leader intron
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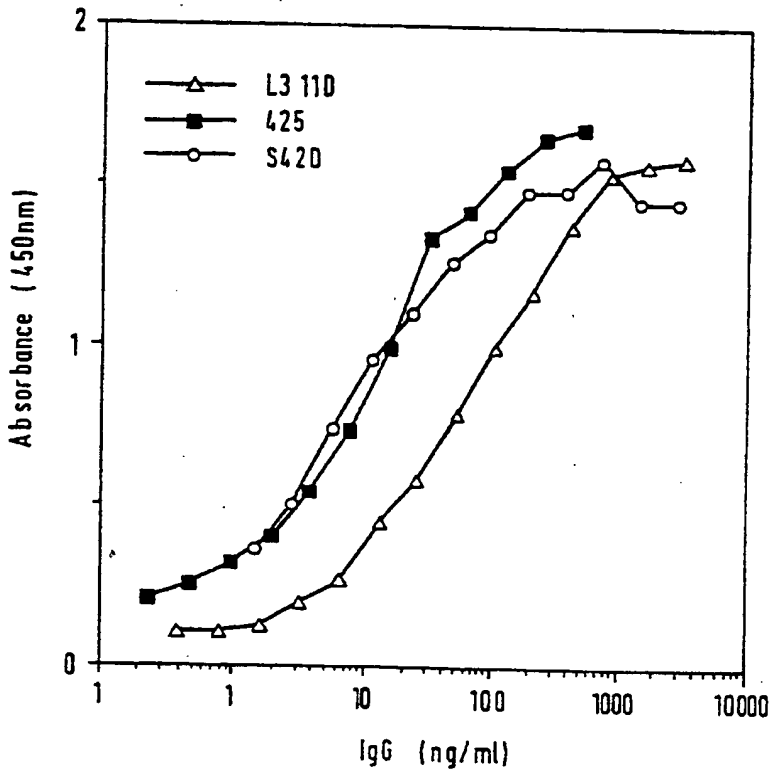
M G W S C I I L F L V A T A T
AGGGGCTCACAGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATGACATCCACTT
Leader SstI XhoI
TGCCTTTCTCTCCACAGGTGTCCACTCCGACATTGAGCTCACCCAGTCTCCAGACAAAGCTC
G V H S D I E L L
BamHI EcoRI
GAGCTGAAACGTGAGTAGAATTTAAACTTTGCTTCCTCAATTGGATCCGAATTC
E L K

Fig. 4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S42D
Absorbance (450nm)
IgG (ng/ml)
0
1
2
1
1
10
100
1000
10000

Figure 5.

(A)

```
GAC ATT GAG CTC ACC CAG TCT CCA GCC TCC CTG GCT GCA TCT GTG GGA     48
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Val Gly
  1               5                  10                  15

GAA ACT GTC ACC ATC ACA TGT CGA GCA AGT GAG AAC ATT TAC TAT AGT     96
Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Tyr Ser
            20                  25                  30

TTA GCA TGG TAT CAG CAG AAG CAA GGG AAA TCT CCT CAG CTC CTG ATC    144
Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile
        35                  40                  45

TAT AGT GCA AGC GCC TTG GAA GAT GGT GTC CCA TCG AGG TTC AGT GGC    192
Tyr Ser Ala Ser Ala Leu Glu Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

AGT GGA TCT GGG ACA CAG TAT TCT TTA AAG ATC AAC AAC ATG CAG CCT    240
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Asn Met Gln Pro
65                  70                  75                  80

GAA GAT ACC GCT ACT TAC TTC TGT AAA CAG ACT TAT GAC GTT CCG TGG    288
Glu Asp Thr Ala Thr Tyr Phe Cys Lys Gln Thr Tyr Asp Val Pro Trp
                85                  90                  95

ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA CGG GCG                327
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
            100                 105
```

(B):

```
CAG GTG CAA CTG CAG GAG TCA GGG CCT GAG CTG GTG AGG CCT GGG GCT     48
Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Arg Pro Gly Ala
  1               5                  10                  15

TCA GTG AAG ATG TCC TGC AAG GCT TCA GGC TAT ACC TTC ACT ACC TAC     96
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr
            20                  25                  30

TGG ATA CAC TGG ATG AAA CAG AGG CCT GGA CAA GGC CTT CAG TGG ATT    144
Trp Ile His Trp Met Lys Gln Arg Pro Gly Gln Gly Leu Gln Trp Ile
        35                  40                  45

GGC ATG ATT GAT CCT TCC AAT AGT GAA ACT AGG TTA AAT CAG AAT TTC    192
Gly Met Ile Asp Pro Ser Asn Ser Glu Thr Arg Leu Asn Gln Asn Phe
    50                  55                  60

AGG GAC AAG GCC ACA TTG AGT GTA GAC AAA TCC TCC AAT AAA GCC TAC    240
Arg Asp Lys Ala Thr Leu Ser Val Asp Lys Ser Ser Asn Lys Ala Tyr
65                  70                  75                  80

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCA ATC TAT TAC TGT    288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
                85                  90                  95

GCA AGA TGG GAC TAC GGT AGT GGC CAC TTT GAC TAC TGG GGC CAA GGG    336
Ala Arg Trp Asp Tyr Gly Ser Gly His Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

ACC ACG GTC ACC GTC TCC TCA                                       357
Thr Thr Val Thr Val Ser Ser
        115
```

Figure 6. (A)

```
GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG      48
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1               5                  10                  15

CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC GAA AGT GTT GAT AAT TTT      96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
            20                  25                  30

GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC     144
Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC     192
Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
    50                  55                  60

AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT     240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

CCT CTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG     288
Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
                85                  90                  95

GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA ATA AAA CGG     336
Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105                 110

GCG
Ala                                                                 339
```

(B)

```
CAG GTG CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGG GCT      48
Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1               5                  10                  15

TTA GTG AAG ATA TCC TGC AAG GCT TCT GGT TAC ACC TTC ACC AGC TAC      96
Leu Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATC     144
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

GGA GAG ATT GAT CCT TCT GAT AGT TAT ACT AAC TAC AAT CAA AAG TTC     192
Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

AAG GGC AAG GCC ACA TTG ACT GTA GAC AAA TCC TCC AAC ACA GCC TAC     240
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
65                  70                  75                  80

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT     288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

GCA AGA TCG GAC TAC GGT AGT AGC CAC TTT GAC TAC TGG GGC CAA GGG     336
Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

ACC ACG GTC ACC GTC TCC TCA                                        357
Thr Thr Val Thr Val Ser Ser
        115
```

Figure 7: (A)

```
GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG     48
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
  1               5                  10                  15

CAG AGG GCC ACC ATC TCC TGC CGA GCC AGC GAA AGT GTT GAT AAT TTT     96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
            20                  25                  30

GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC     144
Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC     192
Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
    50                  55                  60

AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT     240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

CCT TTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG     288
Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
                85                  90                  95

GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGG     336
Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
            100                 105                 110

GCG                                                                 339
Ala
```

(B)

```
GAG GTG CAG CTG CAG CAG TCA GGG GCT GAG CTT GTG AAG CCT GGG GCT     48
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
  1               5                  10                  15

TCA GTG AAG CTG TCC TGC AAG GCT TCT GGC TAC ACC TTC ACC AGC TAC     96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATC     144
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

GGA GAG ATT GAT CCT TCT GAT AGT TAT ACT AAC TAC AAT CAA AAG TTC     192
Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

AAG GGC AAG GCC ACA TTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC     240
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT     288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

GCA AGA TCG GAC TAC GGT AGT AGC CAC TTT GAC TAC TGG GGC CAA GGG     336
Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

ACC ACG GTC ACC GTC TCC TCA                                         357
Thr Thr Val Thr Val Ser Ser
        115
```

Figure 8.

(A)

```
GAC ATT GAG CTC ACC CAG TCT CCA ACC ACC ATG GCT GCA TCT CCC GGG     48
Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Met Ala Ala Ser Pro Gly
  1               5                  10                  15

GAG AAG ATC ACT ATC ACC TGC AGT GCC AGC TCA AGT ATA AGT TCC AAT     96
Glu Lys Ile Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Ser Asn
            20                  25                  30

TAC TTG CAT TGG TAT CAG CAG AAG CCA GGA TTC TCC CCT AAA CTC TTG    144
Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Phe Ser Pro Lys Leu Leu
        35                  40                  45

ATT TAT AGG ACA TCC AAT CTG GCT TCT GGA GTC CCA GCT CGC TTC AGT    192
Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
    50                  55                  60

GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATT GGC ACC ATG GAG    240
Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Gly Thr Met Glu
65                  70                  75                  80

GCT GAA GAT GTT GCC ACT TAC TAC TGC CAG CAG GGT AGT AGT ATA CCA    288
Ala Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ser Ile Pro
                85                  90                  95

CGC ACG TTC GGA GGG GGC ACC AAG CTG GAA ATC AAA CGG                327
Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105
```

(B)

```
GAG GTC AAG CTG CAG CAG TCA GGA CCT GAG CTG GTA AAG CCT GGG GCT     48
Glu Val Lys Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
  1               5                  10                  15

TCA GTG AAG ATG TCC TGC AAG GCT TCT GGA TAC GCA TTC ATA AGT TTT     96
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ile Ser Phe
            20                  25                  30

GTT ATG CAC TGG GTG AAG CAG AAG CCT GGG CAG GGC CTT GAG TGG ATT    144
Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

GGA TTT ATT AAT CCT TAC AAT GAT GGT ACT AAG TAC AAT GAG AAG TTC    192
Gly Phe Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

AAA GAC AAG GCC ACA CTG ACT TCA GAC AAA TCC TCC AGC ACA GCC TAC    240
Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

ATG GAG CTC AGC AGC CTG ACC TCT GAG GAC TCT GCG GTC TAT TAC TGT    288
Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

GCA AGT GGG GAT TAC GAC AGG GCT ATG GAC TAC TGG GGC CAA GGG ACC    336
Ala Ser Gly Asp Tyr Asp Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

ACG GTC ACC GTC TCC TCA                                            354
Thr Val Thr Val Ser Ser
        115
```

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/EP 95/00978

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/28 A61K39/395

According to Inte national Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GENE, vol. 128,no. 1, 1993 AMSTERDAM, THE NETHERLANDS, pages 103-109, L. GARRARD ET AL. 'Selection of an anti-IGF-1 Fab from a Fab phage library created by mutagenesis of multiple CDR loops.' see abstract ---	1-10
X	BIO/TECHNOLOGY, vol. 9,no. 12, December 1991 NEW YORK, NY, USA, pages 1373-1377, L. GARRARD ET AL. 'Fab assembly and enrichment in a monovalent phage display system.' see abstract --- -/--	1-10

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search	Date of mailing of the international search report
23 May 1995	07.06.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Nooij, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 89,no. 10, 15 May 1992 WASHINGTON, DC, USA, pages 4285-4289, P. CARTER ET AL. 'Humanization of an anti-p185HER2 antibody for human cancer therapy.' see the whole document ---	1-10
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INTERNATIONAL SEARCH REPORT

Internat' 'Application No
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